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The environmental issues are now a global concern. Meetings at all levels are being regularly held globally to review the Environmental scenario in an effort to formulate policies, strategies and action plans to eliminate or to minimise the causes of environmental pollution and to use judiciously the existing natural resources in sustainable manner. The major potential Environmental Research areas that we hope will acquire greater and continual importance in the early years of 21st Century are : *Toxicant induced alterations in living organisms, soil, air, water and consequent health risks, Toxicology of agro-chemicals, heavy metals, pharmaceutical products and wastes (industrial, domestic and vehicular) and other toxicants, Microbial toxicology, bioindicators and biotechnology, Ecobiology, biodiversity, forest and wild life conservation, Industrial chemical induced health hazards and occupational diseases, Climate change impact on Environment and consequent health risks, Contamination of underground potable water and human health problems, Safe treatment technologies to combat environmental pollution and related areas.*

Laboratory and field researches provide input for developing strategies, technologies or action plans to conserve natural resources and to repair, maintain and improve the environment from further deterioration. It is with this aim to disseminate the results so obtained by these researches and to make them easily accessible to the scientists and also to the Governments for their proper utilisation, the '*Journal of Environmental Biology*' was launched in the year 1980. In this journey of twenty-two years, the Journal has improved steadily in quality and standard so much so that now it is rated as one of the best Research Journals of the field in the world, covered by most of the Abstracting and Indexing services of the world.

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The help and co-operation rendered by the members of the Editorial Board and the Referees are thankfully acknowledged. It is they who deserve a share of praise for the valuable services they are rendering to the Journal. I thank them all and express the hope that they and readers will continually support this venture.

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(R.C. Dalela)

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Journal of Environmental Biology



January 2003

Volume 24

Issue 01

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Contents

Review article

1. Etiological factors and mechanism involved in relationships between pesticide exposure and cancer.
M. Bounias

Original papers

9. Performance of seed germination and growth of *Vicia faba* L. in fly ash amended soil.
U.N. Rai, D.K. Gupta, M. Akhtar and Amit Pal
17. Environmental stress-induced extracellular isoperoxidase RC3 from rice.
Ki Wan Yi and Mi Young Lee
23. The effects of early experience on subsequent feeding responses in the Tegu, *Tupinambis teguixin* (Squamata : Teiidae).
F. Punzo
29. Dynamics of nitrogen, phosphorus, algal biomass, and suspended solids in an artificial lentic ecosystem and significant implications of regional hydrology on trophic status.
Kwang-Guk An, Seok Soon Park, Kyu-Hong Ahn and Christopher G. Urchin
39. Darkened *Xenopus* tadpoles appeared with neurochemical agents.
Eiji Sato, Kiyoshi Shibata, Yi-Xin Wu, Tadayoshi Uezato, Katsumi Kobayashi and Naoyuki Miura
45. Comparison of growth performance of *Lolium perenne* L., *Dactylis glomerata* L. and *Agropyron elongatum* (Host.) P. Beauv. for erosion control in Turkey.
Ferhat Gökbulak
55. Complex dynamics of toxin producing algal species and primary productivity in two water ponds of Faizabad.
B. K. Dwivedi and G.C. Pandey

63. Effects of rogar and endosulfan on the metabolism of fresh water sponge (*Spongilla lacustris*).
S.T. Ingle, D.N. Shinde and S.B. Namdas
69. Nickel induced changes on some aspects of protein metabolism in the tissues of *Pila globosa*.
M. David, S.B. Mushigeri and M.S. Prashanth
77. A study of neurotoxicity of BHC in relation to residual accumulation on the brain tissue of *Heteropneustes fossilis* (Bloch.).
Ranjit Hazarika
81. Influence of organic wastes and seasonal environmental factors on growth and reproduction of *Eisenia fetida*.
Pulikeshi M. Biradar and Sharabanna D. Amoji
91. Studies on the effect of Isoprocab (MIPC 50 WP) on livestock.
P.R. More, V.P. Vadlamudi, N.M. Degloorkar and S.R. Rajurkar
95. Physico-chemical characteristics of the Vellar estuary in relation to shrimp farming.
M. Rajasegar
103. Role of probiotics on the environment of shrimp pond.
S. Sambasivam, R. Chandran and S. Ajmal Khan
107. Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants.
P.K. Singh and R.K. Tewari
113. Insecticide susceptibility status of *Aedes aegypti* to DDT and dieldrin in desert and non-desert parts of Rajasthan.
S.K. Bansal and Karam V. Singh

Journal of Environmental Biology



April 2003

Volume 24

Issue 02

Website : http://www.geocities.com/j_environ_biol/

Contents

117. Growth and cadmium uptake in barley under cadmium stress.
N.C. Aery and D.K. Rana
125. Physico-chemical and bacteriological investigation on the River Torsa of North Bengal.
Bhaskar Bhadra, Shriparna Mukherjee, Ranadhir Chakraborty and Ashis K. Nanda
135. Gender differences in the metabolism of benzene, toluene and trichloroethylene in rat with special reference to certain biochemical parameters.
Yeshvandra Verma and S.V.S. Rana
141. Response of sugarcane to treated wastewater of oil refinery.
A. Ahmad, A. Inam, Iqbal Ahmad, S. Hayat, Z.M. Azam and Samiullah
147. Longitudinal and seasonal variations of epilimnetic silica in a morphologically complex reservoir and the significance of flow regime and internal processes to their dynamics.
Kwang-Guk An
155. Evaluation of growth potential of Crimean juniper (*Juniperus excelsa* Bieb.) seedlings for the first growing season under Tekir forest nursery conditions in Kahramanmaras, Turkey.
Mahmut D. Avsar and Fatih Tonguc
161. Certain haematological responses in swiss albino mice following exposure to textile dye wastewater.
Neera Mathur, Richa Krishnatrey, Subhasini Sharma, Shipra Pathak and K. P. Sharma
165. Seasonal variations in compostability and production of vermiprotein by *Eisenia fetida*.
M.B. Pulikeshi, S.D. Amoji, U.M. Shagoti and V.A. Biradar

- 173. Utilization of banana agricultural waste: Production of cellulases by soil fungi.**
M.M.V. Baig, V.P. Mane, D.R. More, L.P. Shinde and M.I.A. Baig
- 177. Study of physico-chemical characteristics of water bodies around Jaipur.**
Neera Srivastava, Meena Agrawal and Anupama Tyagi
- 181. Decolorization of anthraquinone dye by *Aspergillus ficuum* in various physiological states.**
Dong Xinjiao and Chen Wenhai
- 187. Effect of industrial effluent on properties of groundwater.**
A. Madhavi and A. Prasad Rao
- 193. Biodiversity of algae and protozoa in a natural waste stabilization pond : A field study.**
N.C. Tharavathi and B.B. Hosetti
- 201. Toxicity of leaf extract of Yellow Oleander *Thevetia nerifolia* on Tilapia.**
S. Sambasivam, G. Karpagam, R. Chandran and S. Ajmal Khan
- 205. Health monitoring of farm labourers engaged in MIPC 50 WP field sprays.**
P.R. More, V.P. Vadlamudi, N.M. Degloorkar and S.R. Rajurkar
- 211. Antimicrobial activities of *Eusteralis deccanensis* and *E. quadrifolia* essential oils.**
J.E. Thoppil, J. Miniya, A. Tajo and M.J. Deena

Journal of Environmental Biology



July 2003

Volume 24

Issue 03

Website : http://www.geocities.com/j_environ_biol/

Contents

Review paper

213. Fungal toxicity with special reference to mycotoxins.
N.K. Bohra and D.K. Purohit

Original papers

223. Impact of urbanization on Bellandur Lake, Bangalore-A case study.
J. S. Chandrashekar, K. Lenin Babu and R. K. Somashekar
229. Determination of a limiting nutrient regulating algal biomass using in situ experiments of Nutrient Enrichment Bioassay (NEB) and empirical relations of nutrients and chlorophyll-a.
Kwang-Guk An
241. Identification of ecologically significant habitats for urban nature conservation : A case study in Turkey.
Fatih Evrendilek
253. Characterization of alkaline phosphatases of some potent phosphate removers.
Anjana Sharma, S. Rajput, D. Khokale and D. Shridhar
261. Bioaccumulation of fenvalerate technical grade in different organs of the frog *Haplobatrachus tigerinus* (Daudin).
K.S. Tilak, K. Veeraiah and L.V.M. Sastry
265. Ultra-structural observations on the lymphoid organs of the freshwater catfish, *Clarias batrachus* (Linnaeus).
Kalpana Dash, K. Saha, A. K. Pandey, A. K. Jain and A. Mukherjee
271. The effect of lead bioaccumulation on haem biosynthetic enzymes in fish.
S.A. Haffor and M. I. Al-Ayed

281. Research of usability of tree leaves and soil in determining the contribution of industry and traffic to air pollution in Bozüyük (Turkey) region.
A. Cicek and A.S. Koparal
289. Effect of kinetin on leaf protein content and its profile in mung bean under salt stress.
Nandini Chakrabarti and S. Mukherji
295. Autecology of the tailed jay butterfly *Graphium agamemnon* (Lepidoptera : Rhopalocera : Papilionidae).
S.P. Venkata Ramana, J.B. Atluri and C. Subba Reddi
305. Hepato and nephrotoxicity in rat exposed to endosulfan.
Nisha Choudhary, Meenakshi Sharma, Pramod Verma and S.C. Joshi
309. Physicochemical and microbiological assessment of Oko-oba – A Nigerian Abattoir.
I.A. Adeleye and A.A. Adebisi
315. Effects of farmyard manure and chemical fertilizers on the nutritional status of the loquat trees.
I. Doran, Z. Kaya and S. Caglar
321. Brainstem auditory evoked responses in young urban and rural boys - A comparison.
Lalan Thakur, J.P. Anand and W. Selvamurthy
327. Effect of the extract of *Thespesia populnea* leaves on mice testis.
P. Krishnamoorthy and S. Vaithinathan
331. Heavy metal pollution in various canals originating from river Yamuna in Haryana.
A. Kaushik, S. Jain, J. Dawra and P. Sharma
339. Hydrobiological study of lake Mirik in Darjeeling Himalayas.
Prithwiraj Jha and Sudip Barat
345. Effect of cybil on reproductive success of wild *Drosophila melanogaster*.
Twinkle Razdan, K.S. Rana and P.N. Saxena
349. Antimicrobial resistance among enteric bacteria, isolated from runoff of the Gangotri glacier, western Himalaya India.
Vinay Singh Baghel, Jaswant Singh and Krishna Gopal

Short communication

357. Sensitivity of newly released varieties of rice to herbicides.
K.P. Singh and N.N. Angiras

Journal of Environmental Biology



October 2003

Volume 24

Issue 04

Website : http://www.geocities.com/j_environ_biol/

Contents

Review paper

359. Insect fauna associated with sugarcane plantations in Sri Lanka
N.C. Kumarasinghe

Original papers

369. Pre-administration of β -carotene protects tissue glutathione and lipid peroxidation status following exposure to gamma radiation
K. Manda and A.L. Bhatia
373. Influence of pH, salt concentration and temperature on the growth of *Aeromonas hydrophila*
G. Vivekanandhan, K. Savithamani and P. Lakshmanaperumalsamy
381. Fresh water fishes as indicators of Kaveri River pollution
T.S. Saravanan, M. Aneez Mohamed, R. Chandrasekar and M. Sundramoorthy
391. Insecticidal activity of the plant *Phyllanthus amarus* against *Tribolium castaneum*
Shalini Khanna, C.N. Srivastava, M.M. Srivastava and Shalini Srivastava
395. Heavy metals alter photosynthetic pigment profiles as well as activities of chlorophyllase and 5-aminolevulinic acid dehydratase (ALAD) in *Amaranthus lividus* seedlings
S. Bhattacharjee and A.K. Mukherjee
401. Effect of mercuric chloride on circulating hormones in adult albino rats
V. Ramalingam, V. Vimaladevi, S. Rajeswary and V. Suryavathi
405. Observations on the histological alterations in various tissues of EUS affected fish, *Channa striatus* (Bloch)
S. A. Mastan and T. A. Qureshi
411. Effects of lead on exploratory behavior and running speed in the shrew, *Blarina brevicauda* (Insectivora)
F. Punzo and C. Farmer
415. Evaluating the seasonal changes of water quality of the Değirmendere and Galyan Rivers (Trabzon, Turkey)
Lokman Altun, Murat Yılmaz, Cengiz Acar, İbrahim Turna, E. Zeki Başkent, Ertuğrul Bilgili

423. *In vitro* effects of metal ions on lipid peroxidation induced by alcohol in mice liver homogenate
Hairong Li, Shaofan Hou, Wuyi Wang, Linsheng Yang, Yonghua Li and Jian'an Tan
429. Landfill leachate-induced toxicity in mice
A. A. Bakare, A. A. Mosuro and O. Osibanjo
437. The effects of compost prepared from waste material of banana plants on the nutrient contents of banana leaves
İlhan DORAN, Bahtiyar ŞEN and Zülküf KAYA
445. Effect of fenvalerate technical grade on acetyl cholinesterase activity in Indian bullfrog *Haplobatrachus tigerinus* (Daudin)
K.S. Tilak, K. Veeraiah, L.V.M. Sastry and J.V. Rao
449. Effect of sodium metabisulphite on germination, growth and yield of *Vigna sinensis*, Savi
M.V. Merlee Teresa, K. Rekha and Alex Bindu
453. Differential effect of cadmium and mercury on growth and metabolism of *Solanum melongena* L. seedlings
P. Neelima and K. Jagannathan Reddy
461. Heavy metal toxicity on dehydrogenase activity on rhizospheric soil of ectomycorrhizal pine seedlings in field condition
T. Ajungla, G.D. Sharma and M.S. Dkhar
465. Dental fluorosis in bovine of Nayagarh district of Orissa
S.K. Maiti, P.K. Das and S.K. Ray
471. Diversity of ground arthropod community at organic and chemically intensive tea plantation of Darjeeling terai
A. Mukhopadhyay, P.W. Sherpa and B. Pradhan
477. Studies on *Merops orientalis* Latham 1801 with special reference to its population in Mayiladuthurai, Tamil Nadu
S. Asokan, K. Thiyagesan, R. Nagarajan and R. Kanakasabai
483. Comparative account of certain enzymes in the serum of homo-iothermal vertebrates subjected to production of myocardial infarction by isoproterenol hydrochloride
Madhavi Gaur and Santosh Kumar
489. Chemical components of heartwood and sapwood of common Yew (*T. baccata* L.)
Gülnur MERTOĞLU-ELMAS
- Short communication
493. Effect of nicotine (plant extract) on sex-ratio of *Drosophila melanogaster* (Meigen)
Shamim Choudhary
495. Author Index Vol. 24 (1 to 4) 2003

Review article

Etiological factors and mechanism involved in relationships between pesticide exposure and cancer.

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Abstract : Professional as well as public exposure to pesticides raise cancer risk. Interaction with adjuvants and with other toxicants increases the actual risk. Endocrine disruption, procarcinogen activation by detoxification enzymes and intercellular communication impairment are involved in the carcinogenic processes. Organochlorine pesticides, including DDT, persist in human tissues for years, with correlated breast cancer incidence. One major point usually underestimated in risk evaluation is the individual variability in detoxification capabilities. Furthermore cellular sites of cancerisation are not necessarily identical to sites primarily exposed to toxicants. Post-diagnostic stress factors and iatrogenic effects of treatment concur to complication of the status of patients. The abnormal increase in the rates of cancer emergence denotes a failure in the application of the precautionary action principle.

Key words : Agrochemicals, Cancer, Endocrine disruption, Individual susceptibility, Precautionary principle.

Introduction

Too few among pesticide studies are dealing with carcinogenicity and epidemiological studies are scarce as compared with efficacy tests. However, former studies emphasized a high human risk from fungicide, herbicide and insecticide exposure (Ferrer and Cabral, 1991; Morgan, 1992; Bruhn *et al.*, 1999; Antle *et al.*, 1995; Iacovo *et al.*, 1999; Burger *et al.*, 2000). This could be related to conflicts of interest, since from 1945 to 1990s, pesticide use has grown by factor 30, less than one percent of sprayed chemicals actually reach the targets (Pimentel, 1995) and crop loss from pests increased by 20% (Ausubel, 1994). Denial of hazard could thus be related to the perspective of repair of prejudice to users and further exposed people, including fetus and mother-fed newborns (Bounias, 2000; Crinnion, 2000). About 175 million people were estimated to be at risk in India (Nigam and Venkatakrishna-Bhatt, 2001).

Epidemiology and individual factors

Epidemiology : A significant increase in chromosomal aberrations has been observed among professional pesticide users in Brazil,

despite protection measures (Antonucci *et al.*, 2000). The genotoxicity requirement for cancer initiation could thus be fulfilled. Then, intermediate processes promoting cancer cell proliferation and distribution in target tissues take place: Metal and organometallic compounds (which include pesticides) induce metallothionein action and further release to extracellular fluids: this results in immune deficiency and inhibition of the elimination of neoplastic cells in remote sites (Lutz, 2000).

Genetical polymorphism and individual risk : Organochlorine detoxification involves mixed function oxidases (CYP450) and glutathione-S-transferases (GST) (Kourourian, 2000). However, either the metabolic transformation may lead to genotoxic and therefore potentially carcinogenic intermediary compounds (Landi, 2000) or the transformation can be incomplete, due to failure of enzymatic systems from saturation by other xenobiotic chemicals or inadaptation to concentrations of the pesticide in the organisms (Iacovo *et al.*, 1999). The latter case associates an increase of organochlorine residues in human body with breast cancer, for instance.

This point emphasizes the problem of individual variability of responses. Genetic

variability was observed for GST among Australian natives (Ilett *et al.*, 2000) and for CYP450 in African-European and American subpopulations (Wandel *et al.*, 2000).

Incidence of GST polymorphism on breast cancer has been evidenced (Sweeney *et al.*, 2000). WHO threshold norms (about 0.005 mg per kg per day) cannot be generalized since : (i) they do not account for individual variations and (ii) at sub threshold concentrations of, say, PAH compounds (Rundle *et al.*, 2000), exposure to even a small excess of another toxic, like a pesticide, is sufficient to break the defenses and give a cancer. It is the role of the precautionary action principle to raise such considerations.

Etiology and molecular mechanisms

DNA adducts : One carcinogenic process involves the binding of chemicals on DNA, resulting in replication and transcription errors, then to mutations and cancer initiations (Seo *et al.*, 2000). Studies on one single compound like DDT sometimes wrongly gives negative results while multiple chemical exposure reveals adduct formation (Lebailly *et al.*, 1998) and cancer initiation (Sellers, 1997).

Alteration of the intercellular communication : The pioneering works of Trosko (1990, 1998) have identified the causal mechanisms leading from impairment of intercellular communication to cancer (Bounias, 2000). This is exemplified by DDT and related compounds, which increase, cell proliferation and decrease the expression of connexin, a substance involved in cellular communication, in hepatic cells (de Graaf *et al.*, 2000). This study emphasized insufficient precaution in experimental protocols can mask toxicity and confirmed the implication of pesticides like DDT in non-genotoxic ways of carcinogenicity.

Hormonal mechanisms : Breast cancer rates vary as the tissue concentrations of organochlorine, though statistical risk levels can be weakened by still poorly identified factors in people not professionally exposed (Burger *et al.*,

2000). Among these factors, endocrine disruption is involved for breast cancer through the progesterone receptor family (Raman *et al.*, 2000). Such mechanisms involve the immune system under estrogenic humoral control for the detection and destruction of cancer nodules (Mor *et al.*, 2000). Estrogenic and antiestrogenic effects, as typical of endocrine disruptor activity, have been evidenced for many herbicides, fungicides, insecticides, nematocides and a host of other compounds (Bruhn *et al.*, 1999). In this family, DDT stands for both organohalogen and polychlorinated biphenyl derivative.

Endocrine – carcinogenic interactions :

Detoxification enzymes of the cytochrome P450 type have contradictory effects : on one hand they destroy toxic compounds, but on the other hand they can activate procarcinogens into carcinogens, which occurs with estrogens and other hormonal substrates (Shimada, 2000). Cross induction of CYP450 results from exposure to different toxicants, therefore, endocrine-disrupting pesticides could be classified as potentially related to pro-carcinogenicity. A list of carcinogenic and endocrine-disrupting pesticides, given in Table 1, shows some consistency of both of these activities for various pesticides.

The particular case of DDT

Introduction : DDT was matter of continued toxicological research for the last 40 years. Its economical importance resulted from the orientation of human activities to systematic chemical control of pests in the middle of XXth century. Though use of DDT was banned from the 1970s in many countries, its residues, including those of its most common metabolites DDE and DDD, are still commonly found in food, animals and humans, due to its persistence, spreading and clandestine use (Bounias, 2000).

Endocrine effects : Cancer results from DDT-induced break of estrogenic regulation (Robison *et al.*, 1985; Guttes *et al.*, 1998). The mechanism involves a structural alteration of adrenals (Chowdhury *et al.*, 1990; Jonsson *et al.*, 1992) and action on estrogen receptors with possibility

of delayed risk from prenatal exposure (Metcalf *et al.*, 2000). Since analyses are usually performed in people not particularly exposed, the following observations should be considered as a lower boundary of what would be expected from professional exposure.

Persistence of residues in humans : Among many other reports, it was recently shown that : (i) Indonesian women milk contains DDT residues far above formerly detected levels (Shaw *et al.*, 2000) and (ii) there exists relationships between fat body DDT concentrations and breast cancer (Bagga *et al.*, 2000).

General toxicity of DDT : The recently evidenced DDT toxicity to hepatic cells (Rhouna *et al.*, 2000) suggests impairment of hepatic detoxification systems. Moreover, weak DDT doses alter corticosteroid patterns, which could impair the response to stress. This in turn may worsen the secondary effects of anticancer antibiotics, by enhancing the generation of toxic oxygen radicals (Bounias *et al.*, 1997).

Endocrine disruption by DDT and other organohalogen compounds is documented (Metcalf *et al.*, 2000) and likely results in observed damage to wildlife (Seba and Joy, 2000). Unfortunately, the simultaneous administration of agonists of the estrogen family does not suppress estrogenic effects of DDT (Greenlee *et al.*, 2000).

Regarding intercellular communication, the expression of connexin is inhibited by DDT, which in addition dose-dependently increases cellular proliferation (deGraaf *et al.*, 2000). Thus, DDT stands for a typical candidate for carcinogenicity.

Ambiguities in the concept of no-effect thresholds

Multiplicity of toxic factors : Multiple chemical exposure tend to saturate the systems of detoxification : then, even a very small dose of an additional toxicant can result in severe toxicity. On the other hand, many toxicants are metabolized into products that are more toxic.

Such "worsening detoxification" processes are documented for chloroform, chlorobenzenes, benzopyrenes, parathion, and other organophosphorous compounds, phenolic derivatives, in particular chlorophenols, nitrosamines and miscellaneous pesticides (Bounias, 2000). This adds to the problem of individual variability. The threshold phenomenon is a non-linear function of a very large number of variables, so that the limits of tolerance to prolonged exposure to toxicants decrease with the duration of exposure, the nature and diversity of chemicals, the individual status of people's health and features, so that only prevention can be a efficient measure.

Again, the precautionary action principles stand for the major if not only solution.

A typical dose-responses : Biphasic responses are common features of the normal regulation pathways, and their importance has been underestimated in toxicology. These mechanisms can hide specific noxious effects at very low doses (Bounias, 1990; Bounias *et al.*, 1990).

The masking of causality parameters : Clinical effects of intoxicants can manifest in various forms depending on the delay from exposure. This has been documented for organophosphorous pesticides, where low levels of chronic exposure can result in sub clinical symptomatic effects at short term, followed by severe chronic effects at long term (Lieberman, 2000).

Most of these questions still are poorly addressed, though damage to professional populations has been evidenced for long (Antle and Pingali, 1995).

Neuropsychical vs. iatrogenic factors

Psychical stress resulting from various situations of distress favours the emergence of cancer (Andrews *et al.*, 2000). Furthermore, the announcement of the diagnostic and the prescription of anticancer therapies generate endocrine perturbations like correlative increases of cortisol and met-enkephalin in patients with breast cancer (Kajdaniuk *et al.*, 2000), while the

patients have also to fight against iatrogenic effect of prescribed drugs (Chang, 2000). Among

noxious side effects, cognitive troubles, alterations

Table – 1 : A non-exhaustive list of pesticides classified as carcinogens or potentially carcinogens (wherever genotoxic or not), and as endocrine disruptors or likely such, with putative involvement in carcinogenicity. Data come from Huff *et al.* (1988), Montesano *et al.* (1988), Smith (1988), Huff *et al.* (1991), Kitchin *et al.* (1994), Fung *et al.* (1995), Bruhn *et al.* (1999), Crinnion (2000), Boros *et al.* (2001).

Classes of pesticides	Carcinogens and potential carcinogens	Endocrine disruptors	Possibly endocrine disruptors
Insecticides : organo-P	Parathion; Methyl-parathion; Demeton; Fenthion; Isofenphos	Parathion; Chinalphos; Dicofol;	Dimethoate; Phosmet;
Carbamates	Malathion; Metepa; Phosphomidon	Carbaryl; Aldrin; Chlordane; Nonachlor	Aldicarb; Methomyl; Oxychlordane
Organoc-Cl	Carbaryl Aldrin; Chlordane; Dieldrin; DDT; DDE; Dichlorvos; Endrin; Heptachlor; lindane; Mirex; Tetrachlorvinphos;	β -HCH γ -HCH (Lindane)	Heptachlor Mirex
Miscellaneous	Toxaphene 1, 3-dichloropropene Trichlorfon; Dibromure de methyle	Pyrethroids;	
Herbicides	Amitrole; Diallate; Monuron Nitrofen; Sulfallate	Atrazine; Amitrol; 2; 4; 5 TP; Metribuzin; nitrofen; Trifluralin;	Alachlor;
Fungicides	Captain; Chlorothalonil; HCB; O-phenyl phenol; Quintozene; Ziram	Fenarimol; HCB; Mancozeb; Maneb; Metiram; PCP; Thiram; Ziram;	Benomyl; Guazatin; Tridemorph;

of thinking processes, memory and concentration deficit have been observed (Bredzen *et al.*, 2000).

Prior to the cancer stage, victims of pesticide intoxication may have already experienced neurotoxicity symptoms, irritability, confusion, tremor, head aches, nausea, vomiting, hypersensitive hyperesthesia, and other symptoms like respiratory depression, cardiac arrhythmia, aplastic anaemia, porphyria cutanea tarda. For example, DDT induces fatigue symptoms with electromyographic signature, cognition troubles,

vision trouble and ataxia (Kailin and Hastings, 1966 a & b).

Therefore, neuro – psycho – toxicology, a new paradigm (Bounias *et al.*, 1998), should be emphasized in modern toxicology. Its role should be particularly important in recidive cases.

Discussion

Analysis of direct causality : The authors of the abovementioned existence of a direct causation of breast cancer by DDT mitigated their conclusions by raising the fact that adjustment for patient's

age weakened the statistical levels of significance of the results. This argument may however be invalid. In effect, generally : (i) the age of patients during exposure is not homogenous; (ii) exposure to other toxicants is not assessed; (iii) the delay from nodule formation and its eventual evolution to clinical cancer signs is not a constant; (iv) the distribution of individual susceptibility factors of exposed people is not known and does not linearly vary with subjects age. Therefore, it may be scientifically incorrect to simply adjust data to age classes, which in contrast could in some situations hides causality relationships.

Weakening of risk assessment methods :

Industrial lobbying press authorities to decrease the cost of toxicological studies (Morelli, 2000) and it has been proposed to switch the risk assessment to a survey of residue threshold levels (Kroes *et al.*, 2000). This weakening of toxicological survey was preceded by attempts for keeping only the mutagenic activity and DNA repair testing, at the expense of the detection of promoters and proliferators, which has been shown to be a dangerous trends (Bounias and Bonaly, 1995 a & b). DNA repair testing is also jeopardized by individual variability (Berwick and Vineis, 2000) and the mutagenic initiation of the carcinogenic process is in all cases just the beginning of a complex cascade of phenomena (Zeiger, 2000). Even structure-activity studies only recently started to incorporate factors related to the biological target themselves (Sello *et al.*, 2001).

Ethical views and the precautionary action

principle : In France, the rate of emergence of all cancer types has doubled from 1970 to 1990 (Vue-Desingue, 1996) despite growing efforts of medical research to find anticancer therapies. This should call attention on the need to eliminate etiologial and causative factors, including, as acknowledged by the U.S. National Institute of Environmental Health (NIEH, 2000) to avoid exposure to risk factors, of which pesticides represent an important part. The precautionary action principle recommends to spend less by performing efficient preventive orientation of

human activities so as to avoid spending more in low to inefficient curative action, even if the later represent a huge economical market, that is a market profiting from people's distress. Two attitudes deserve attention : (i) it has been claimed that huge amounts of money can be invested in such ethically dangerous research as Genetical engineering, even if only a small number of genetically diseased patients would benefit from these researches; (ii) tolerance to pollutant exposure actually allows a proportion of population (usually from 10^{-6} in the 1960s, down to about 10^{-4} nowadays) to be the regular victims of economical activities. In India, recent studies have estimated the number of people professionally and publicly exposed to pesticide hazard to reach about 173 million (Nigam and Venkatakrishna-Bhatt, 2001). Assuming a rate of one victim every 100000 exposed, this would result in 1730 persons sacrificed to economical considerations. In the first case one expects a small number of expensively treated persons, while conversely, in the second a large number of persons are sacrificed upon low cost.

It is the role of the precautionary action principle to induce human activities to shift towards alternative management of Planet Earth that would preserve equitable distribution of wealth among the entire living community without which humans would not survive. Further laws should be elaborated within the window of this perspective.

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Performance of seed germination and growth of *Vicia faba* L. in fly ash amended soil.

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Abstract : The performance of *Vicia faba* L. in soil amended by different concentrations of fly ash has been studied. The parameters considered are seed germination, growth behaviour and nodulation frequency of the plant. Results revealed that while fly-ash amendment to the soil improved the growth performance at initial stages with application of lower concentrations, it was inhibitory at higher exposure concentrations. Although there was no difference in survival rates, but the seedling growth was reduced in comparison to control plants. Fly ash delayed the nodulation as lesser number of nodules was recorded at higher amendments. Results suggested feasibility of growing *V. faba* in fly ash contaminated area.

Key words : Amended soil, Fly ash, Seed germination, *Vicia faba*.

Introduction

Although coal has emerged as one of the sustainable energy source and being used for power generation, the disposal of fly ash, the combustion residue has created a variety of environmental problems (Adriano *et al.*, 1980). Fly ash contains a high concentration of toxic metals (Al, B, Mo, Cu, Zn, Cd, Hg, As, Pb, etc.) along with low nitrogen and phosphorus content and pH ranged between 4.5-12.0 depending on the S content of parent coal. Toxic metals present in fly ash enter into the ecosystem out of ingestion through water, the food-chain biomagnification or inhalation and produce high order effects on mankind. The alkaline nature of fly ash prompted in its use as soil limiting material in agriculture production (Phung *et al.*, 1978; Terman *et al.*, 1978, Marsh and Grove, 1992, McCarty *et al.*, 1994). However, its application in agriculture on a large scale is still limited (Furr *et al.*, 1975; Ciravolo and Adriano, 1979). Some plants have been able to grow on fly ash amended soils without any manifestation of injury symptoms (Aitken and Bell, 1985; Singh *et al.*, 2000). In contrast, some reports suggest that small applications of fly ash in agricultural fields are suitable for better crop management (Gutenmann *et al.*, 1976; Furr *et al.*, 1978; Singh *et al.*, 1997).

Besides, growth trials made on grains and vegetable crops in fly ash amended soils achieved some success (Aitken and Bell, 1985; Wong and Wong, 1990). In addition, legume plants, symbiotic N₂-fixing bacteria, and blue-green algal biofertilizer have been reported to improve nitrogen and phosphorus contents of infertile and toxic fly ash (Rai *et al.*, 2000; Cheung *et al.*, 2000).

The present investigation was planned to assess the feasibility of fly ash amended soil for better growth of *V. faba* and to compare the difference between growth performance and tolerance between different fly ash amendments. Further, effect of these amendments on nodulation frequency of the plant was also studied.

Materials and Methods

The fly ash used in this study was collected from National Thermal Power Corporation, Unchahar, Raebareli, U.P. Plants of *Vicia faba*, commonly known as 'Broad Bean' or 'Field Bean' or 'Bakla' belonging to the family leguminosae has been selected for the present study. The plants forming ridges on both sides when the plants are about 15 cm tall or when they are ca 30 cm tall, the stems are earthen up and supported with sticks, growth between the 4th and

9th week after sowing is the critical period of phosphorus requirement.

The fly-ash samples collected from the fly-ash pond at Unchahar were oven dried for 7 days. Nitrogen was determined following microkjeldal technique, K and Ca by flame photometry and calorimetric methods. The pH and electrical conductivity (EC) were determined by pH meter and conductivity meter, respectively using fly ash distilled water with a ratio of 1 : 25 (Piper, 1966).

The methods and formula applied for the estimation of toxic metals was followed from the manual of Perkin Elmer (Analytical Method for Atomic Absorption Spectrophotometer). Fly-ash sample (100 mg) was digested in concentrated HNO_3 : HClO_4 (v/v, 3 : 1) at low temperature until a clear solution was obtained.

Seed germination was recorded in 25%, 50%, 75% and 100% fly-ash condition for 2, 3 and 5 days. Leaf area (cm^2) as an index of plant growth was measured with an area measurement system (Delta t Devices, U.K.). Leaf samples were analyzed for chlorophyll (extraction) in 80% acetone (v/v) solution and estimated according to

Machlachlan and Zalik (1963). Carotenoid was estimated following Duxbury and Yentsch (1956).

Plants of *V. faba* were uprooted at different time intervals and the number of nodules present on the root is counted with the help of a hand lens.

Results

The general physico-chemical properties of fly ash and the garden soil used in this study has been summarized in Table 1. The results revealed that fly-ash has a high pH and low in total nitrogen and phosphorus content and was enriched with high levels of toxic metal like Cu, Mn, Zn, Fe, Ni and Pb. The garden soil used as ameliorane and control during the experiment has alkaline pH, but has sufficient amount of nitrogen and phosphorus for supporting plant growth. Besides, it has slightly higher concentration of these metals.

The data presented in Figure 1 shows the effect of different fly ash concentration on the percent germination, which reduced significantly, and it was almost 30% in 100% fly-ash condition as compared to control after 5 days. However, a

Table – 1 : Physico-chemical properties of fly ash and garden soil used in the study.

Parameters	Fly-ash	Soil
pH	9.2	8.0
Electrical conductivity (m mhos cm^{-1})	0.61	0.04
Cation exchange capacity meq (100 g^{-1})	1.26	7.32
Total nitrogen (%)	0.02	0.08
Total phosphorus (%)	0.02	0.03
Organic carbon (%)	1.17	1.35
Cu (mg g^{-1})	39.5	36.2
Mn (mg g^{-1})	14.1	10.3
Zn (mg g^{-1})	77.2	55.3
Fe (mg g^{-1})	245	250
Ni (mg g^{-1})	141	17
Pb (mg g^{-1})	40	8.5

comparative examination of the data showed that the seed germination was more in 100% fly ash at initial days, which was significantly inhibited at later stages of seedling growth.

The plants of *V. faba* growing in different combination of fly ash and soils showed a reduction in growth. The root and shoot length of the plant is affected and the morphological

appearance of the plant has been changed. The data shown in Table 2 further revealed that by increasing fly-ash level from 25% to 100% there

was a drastic reduction with reference to these parameters. The number of leaves per plant and the leaf area were also affected by fly-ash

Table - 2 : Effect of fly ash on biomass and plant height of the *V. faba*

Parameters		Treatment duration (d)		
		15	30	45
(A) Biomass (g DW)				
whole plant	Control (soil)	19.22±1.32	20.14±1.23	22.32±1.42
	25% fly-ash	8.44±0.95	9.91±0.98	10.71±1.02
	50% fly-ash	17.81±1.65	18.18±1.36	19.41±1.39
	75% fly-ash	18.20±1.45	18.42±1.42	19.11±1.45
	100% fly-ash	9.93±0.65	10.22±0.95	10.54±0.99
Root	Control (soil)	5.22±0.45	5.55±0.49	5.56±0.48
	25% fly-ash	4.44±0.36	5.15±0.42	5.70±0.52
	50% fly-ash	4.67±0.42	4.88±0.47	4.20±0.39
	75% fly-ash	2.55±0.32	2.66±0.35	2.69±0.37
	100% fly-ash	2.69±0.42	2.78±0.44	2.88±0.56
Shoot	Control (soil)	9.99±1.3	10.44±1.5	11.97±1.7
	25% fly-ash	2.65±0.45	3.31±0.61	3.44±0.64
	50% fly-ash	9.96±0.76	10.02±0.73	11.21±0.82
	75% fly-ash	10.85±0.89	10.94±0.91	11.40±0.97
	100% fly-ash	5.03±0.23	5.13±0.34	5.24±0.41
Leaf	Control (soil)	4.00±0.16	4.14±0.19	4.77±0.41
	25% fly-ash	1.34±0.63	1.48±0.68	1.55±0.70
	50% fly-ash	4.17±0.21	4.27±0.26	4.00±0.18
	75% fly-ash	4.80±0.22	4.80±0.21	5.00±0.27
	100% fly-ash	2.19±0.17	2.29±0.23	2.40±0.32
(B) Plant length (cm)				
total plant	Control (soil)	25.0±1.38	28.0±1.47	34.5±1.72
	25% fly-ash	17.0±1.05	20.0±1.21	22.0±1.43
	50% fly-ash	26.0±2.0	30.0±2.31	32.0±2.42
	75% fly-ash	29.0±2.15	31.0±2.33	32.0±2.28
	100% fly-ash	28.0±2.22	32.0±2.37	34.0±2.42
Root length	Control (soil)	5.0±0.56	6.0±0.62	6.5±0.71
	25% fly-ash	2.0±0.13	5.0±0.32	7.0±0.43
	50% fly-ash	5.0±0.45	6.0±0.59	6.0±0.60
	75% fly-ash	5.0±0.32	7.0±0.62	7.0±0.61
	100% fly-ash	5.0±0.42	6.0±0.59	7.0±0.61
Shoot length	Control (soil)	20.0±1.36	22.0±1.42	28.0±1.53
	25% fly-ash	15.0±1.05	15.0±1.04	15.0±1.05
	50% fly-ash	21.0±1.32	24.0±1.42	26.0±1.51
	75% fly-ash	22.0±1.49	24.0±1.42	25.0±1.49
	100% fly-ash	23.0±1.12	26.0±1.57	27.0±1.52

supplementation into the soil and it was maximum in 100% fly ash grown plant. Different fly ash combinations also affected root, shoot and leaf biomass at different harvesting period and the toxic effect was maximum after 45 d of growth. Fly ash not only affected the growth of the plant

but also had a great influence on the nodulation of the plant, which are considered the site of N_2 -fixation. Although nodules were inhibited in various fly ash supplemented soil, however, 100% fly ash supported only 2% nodulation in the plant.

The result presented in Table 4 showed that there was an initial increase in chlorophyll content of the plant, which reduced slightly after 45 days of growth. However, the value of

chlorophyll in fly ash treated plants was always more than the control plant growing in garden soil. It was approximately 1.79 mg g^{-1} fw in control, which increased to 1.90 in 100% fly ash

Table - 3 : Effect of fly ash on photosynthetic area (leaf area) and nodule number of the *V. faba*.

Parameters	Treatment duration (d)		
	15	30	45
(C) Leaf area (cm^2)			
Control (soil)	1.37 ± 0.23	1.55 ± 0.32	1.82 ± 0.38
25% fly-ash	0.83 ± 0.05	0.84 ± 0.06	0.90 ± 0.08
50% fly-ash	1.15 ± 0.5	1.38 ± 0.8	1.39 ± 0.9
75% fly-ash	1.09 ± 0.32	1.18 ± 0.36	1.75 ± 0.43
100% fly-ash	1.16 ± 0.46	1.41 ± 0.49	1.51 ± 0.52
(D) Number of leaves			
Control (soil)	48.0 ± 2.5	49.0 ± 2.6	52.0 ± 2.8
25% fly-ash	16.0 ± 1.23	18.0 ± 1.31	19.0 ± 1.34
50% fly-ash	14.0 ± 1.16	15.0 ± 1.18	19.0 ± 1.32
75% fly-ash	14.0 ± 1.05	15.0 ± 1.12	17.0 ± 1.27
100% fly-ash	14.0 ± 1.08	20.0 ± 1.14	23.0 ± 1.47
(E) Nodule number			
Control (soil)	0.0	0.0	98.0 ± 3.0
25% fly-ash	0.0	0.0	64.0 ± 2.8
50% fly-ash	0.0	0.0	51.0 ± 2.6
75% fly-ash	0.0	0.0	42.0 ± 2.5
100% fly-ash	0.0	0.0	2.0 ± 0.46

treated condition. In contrast to chlorophyll content, carotenoids content reduced significantly in different fly ash treated condition. The effect on carotenoid is evident from very beginning of experiment i.e., 15 d of treatment and about 30% inhibition in carotenoid content was found in 100% fly ash.

All naturally existing elements can be found in fly ash (Klein *et al.*, 1975) and is substantially enriched in trace elements compared with the parent coal. Fly-ash pH and electrical conductivity (EC) were 9.2 and 0.61, respectively. However, fly ash was typically low in N (0.02%) as compared to soil (0.08%). High alkalinity of fly ash might be due to the presence of high concentration of oxides of Ca and Mg, which form hydroxides in presence of water (Furr *et al.*, 1975). Since EC is a measure (indirect) of total ions, the enrichment of fly ash with various essential and non-essential cations and anions can appreciably increase its EC.

With regards to seed germination, it was significantly reduced even at lower fly-ash application rate and no sign of stimulation was found at any of the growth stages. Since the fly ash contains many growth essential elements like K, Ca, Mg, Fe, Zn, B, Mo and S, etc., it might induce seed germination at lower rates of application. However, at higher application rates, as in 100% fly ash, trace elements like Cu, Co, Ni, Se, Al, Cr, etc. present in the fly ash might impair seed germination process and so, either delayed or inhibited the process (Wong and Bradshaw, 1981; Vollmer *et al.*, 1982). The plants of *V. faba* growing on different combinations of fly-ash and soil might have accumulated different proportions of toxic metals leading to reduced growth of the plant.

Various investigators (Aubert and Pinta, 1977; Page *et al.*, 1979; Moliner and Street, 1982; Mishra and Shukla, 1986) have reported the enrichment of soils and plants with trace elements

by fly-ash application. However, metal uptake by the plant from the soil depends upon the concentration of metal in the soil and on the physiological requirement for the metal by the

plant (Singh *et al.*, 1997). Plants of *V. faba* growing on different combinations of fly ash when compared to the plant growing in normal soil showed a significant reduction in plant height,

Table – 4 : Chlorophyll and carotenoid (mg g^{-1} FW) content in *V. faba* as affected by different combinations of fly ash with soil at different harvesting period

Parameters		Treatment duration (d)		
		15	30	45
Chlorophyll a	Control (Soil)	1.13 \pm 0.10	1.38 \pm 0.29	1.38 \pm 0.29
	25% fly-ash	1.46 \pm 0.30	1.04 \pm 0.10	0.95 \pm 0.14
	50% fly-ash	1.41 \pm 0.10	1.69 \pm 0.19	1.81 \pm 0.22
	75% fly-ash	1.44 \pm 0.11	1.71 \pm 0.19	1.89 \pm 0.20
	100% fly-ash	1.60 \pm 0.12	1.80 \pm 0.19	1.98 \pm 0.22
Chlorophyll b	Control (Soil)	0.40 \pm 0.02	0.41 \pm 0.10	0.41 \pm 0.40
	25% fly-ash	0.56 \pm 0.14	0.35 \pm 0.42	0.34 \pm 0.05
	50% fly-ash	1.25 \pm 0.09	0.88 \pm 0.45	0.88 \pm 0.14
	75% fly-ash	0.55 \pm 0.14	0.89 \pm 0.55	1.19 \pm 0.10
	100% fly-ash	0.66 \pm 0.18	0.98 \pm 0.68	1.20 \pm 0.11
Total chlorophyll	Control (Soil)	1.54 \pm 0.12	1.79 \pm 0.20	1.79 \pm 0.20
	25% fly-ash	2.00 \pm 0.45	1.37 \pm 0.15	1.27 \pm 0.19
	50% fly-ash	1.51 \pm 0.12	1.66 \pm 0.18	1.78 \pm 0.20
	75% fly-ash	2.12 \pm 0.35	1.79 \pm 0.19	1.87 \pm 0.21
	100% fly-ash	1.98 \pm 0.48	1.80 \pm 0.14	1.90 \pm 0.24
Carotenoid	Control (Soil)	7.53 \pm 0.91	7.83 \pm 0.61	7.85 \pm 0.61
	25% fly-ash	3.45 \pm 0.41	3.88 \pm 0.48	5.98 \pm 0.55
	50% fly-ash	4.44 \pm 0.81	6.82 \pm 0.67	6.92 \pm 0.77
	75% fly-ash	5.33 \pm 0.98	6.66 \pm 0.93	8.33 \pm 0.91
	100% fly-ash	4.98 \pm 0.75	6.25 \pm 0.84	7.27 \pm 0.74

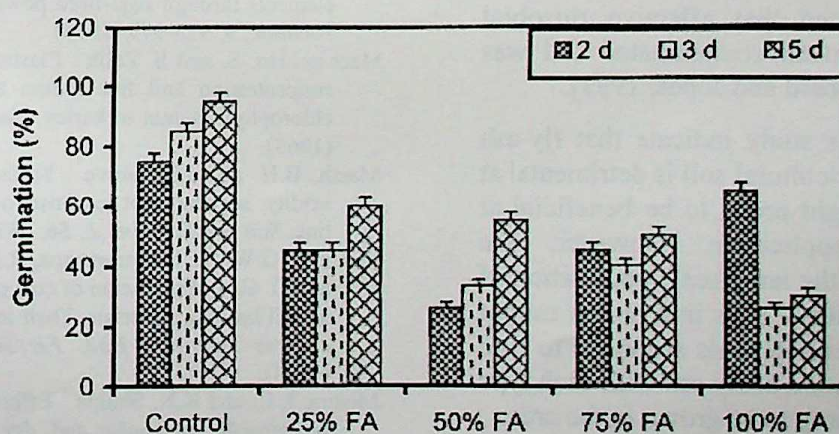


Fig. 1 : Effect of different concentrations of fly ash (FA) on seed germination in *V. faba*.

number of leaves and general vigor of the plant. The lower biomass of the plant in different treatments of the fly ash might be due to accumulation of toxic metals coupled with desirable changes in the property of soil. This is in

agreement with our earlier report on a leguminous tree plant (Vajpayee *et al.*, 2000). Our results showed an enhancement in chlorophyll content at initial treatment duration, which decreased at later stages of plant growth. Such lower level of

pigment content in 25% fly-ash grown plant may be either due to reduced synthesis or accelerated degradation, a decrease in chlorophyll content during metal supply has been reported (Prasad and Prasad, 1987). Associated with initial steps in the chlorophyll synthesis is a metal sensitive enzyme, δ -aminolevulinic acid dehydratase (ALAD). Like the chlorophyll content, the activity of this enzyme decreased in the leaves of *Pennisetum typhoideum* and *Phaseolus vulgaris*, when exposed to higher level of Pb, while the contents of δ -aminolevulinic acid (ALA) was unchanged (Prasad and Prasad, 1987). The decreased activity was supposed to be due to an interaction of Pb (and other element) with -SH groups at the active site of the enzyme. It may likely that the carotenoid content of the test plant might have affected in a similar way under fly ash supplemented conditions.

Since fly ash contains different type of toxic metals, which are not essential for plant growth, it inhibited nitrogenase activity of the plant resulting into unfunctional nodules. The data presently obtained showed that fly ash had a great influence on initiation and establishment of nodules. Only lower application of fly ash allowed nodules initiation in the plant, however, nothing could be concluded about the functioning of nodules in absence of enzymatic data. Further, it has been suggested that effective rhizobial population size in metal contaminated soil was less than control (Obbard and Jones, 1993).

Over all, this study indicate that fly-ash application to the agricultural soil is detrimental at higher doses and might prove to be beneficial at lower rates of application. However, the recommendation for the large-scale application of fly ash to the agricultural soils in a region cannot be made, unless extensive trials are made to find out a proper combination of fly ash with each type of soil and for each crop to be grown in the area.

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Fly ash amended soil on seed germination and growth.

15

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Environmental stress-induced extracellular isoperoxidase RC3 from rice.

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Abstract : Effects of various environmental stresses such as heavy metals, salts and low (high) temperature on the secretion of peroxidase isozyme into the medium were examined in rice (*Oryza sativa* cv. Nak-Dong) suspension culture. The major extracellular peroxidases secreted into the medium by various stresses were cationic isoperoxidases. A far migrating cationic isoperoxidase RC3 was isolated from the medium after application of CaCl_2 , the effective stimulator for peroxidase secretion. Isolation of extracellular isoperoxidase RC3 was accomplished by ammonium sulfate fractionation, CM-cellulose cation-exchange chromatography, and Sephacryl S-100 gel filtration. The enzyme was a glycoprotein having molecular weight of approximately 34 KDa as determined by SDS-PAGE and 38 KDa by Sephacryl S-100 gel filtration. The pI value of the enzyme was 8.9. Kinetic studies revealed that the optimum pH of the enzyme was 6.0 for guaiacol and H_2O_2 , and the K_m values for guaiacol and H_2O_2 were 10.5 mM and 3.2 mM, respectively.

Key words : Environmental stress, Extracellular isoperoxidase, Rice (*Oryza sativa* cv. Nak-Dong).

Introduction

Plants contain abundant amounts of multiple peroxidases [EC 1.11.1.7] that exhibit broad substrate specificities (Hiraga *et al.*, 2001). Plant peroxidases are associated with many reactions including lignification, indole-3-acetic acid oxidation, cell wall polysaccharide cross-linking and wound healing (van Huystee and Cairns, 1982). There are also evidences that increase in peroxidase activity can appear as a metabolic response to various environmental stresses (Fang and Kao, 2000). Multiple forms of peroxidase isozymes were isolated from many higher plants including Korean radish, rice and Arabidopsis (Lee and Kim, 1994; Lee *et al.*, 1994; Tognolli *et al.*, 2000; Lee *et al.*, 2001). Their enzymatic properties and physiological functions were also examined at the protein and gene level (Dunford, 1999; Hiraga *et al.*, 2000). In case of rice (*Oryza sativa* L.), four peroxidase components from green leaves and two forms of peroxidases were isolated and characterized (Ito *et al.*, 1991; Padiglia *et al.*, 1995). Rice cationic peroxidase, PO-C1, which was induced in

incompatible interactions between the vascular pathogen *Xanthomonas oryzae* pv *oryzae* and rice, was also purified (Young *et al.*, 1995). Moreover, ascorbate peroxidase was suggested as a cellular protectant in the etiolated rice shoots in terms of proteomic profiling (Komatsu *et al.*, 1999). One of the main functions of peroxidases in various sources is related with the defense system, ensuring the detoxification of the reactive oxygen species (Higa *et al.*, 2001). Moreover, the changes of peroxidase activity and isozyme patterns have been reported to be involved in the influence of different environmental factors, including the metal ion effect (Fang and Kao, 2000), salt effect (Bakardjieva *et al.*, 1996) and air pollution damage (Castillo *et al.*, 1987; Lee, 2002). In this respect, many authors began to find information concerning the metabolic response of plants to different stress factors in suspension cultures. Cell suspension cultures provide a convenient system for investigating stress-induced changes of enzyme at the cellular and extracellular secretory level. Usually plant cell cultures produce various enzymes and secrete some of them into the medium, however, very little information is

available on the effectors on which peroxidase secretion into the medium is dependent.

The present study describes the alterations of peroxidase secretion into the medium by various environmental stresses. Isolation of extracellular isoperoxidase RC3, the major secretory isoperoxidase, by using several chromatographic methods was investigated. Characteristics of the physico-chemical properties of extracellular isoperoxidase RC3 were also investigated.

Materials and Methods

Experimental plant

A rice callus line (*Oryza sativa* cv. *Nak-Dong*) was maintained routinely in AA2 media. The rice cell suspension culture of 20 ml was aseptically transferred to 80 ml of AA2 medium in a 250 ml flask and then grown for 14 days (Lee, 1997).

Peroxidase activity assay

The peroxidase activity with guaiacol as a substrate was assayed by the procedure of Lee and Kim (1994). The assay mixture contains 40 mM phosphate buffer, 15 mM guaiacol, 5 mM H_2O_2 and 50 μ l of enzyme preparation in a total volume of 1 ml. The increase in absorbance at 470 nm was measured.

Effects of various environmental stresses on the secretion of peroxidase

The effects of various stresses on the secretion of rice peroxidase were examined by comparing the specific activity of cellular peroxidase with that of extracellular peroxidase in the medium. The concentrations of heavy metals, such as $NiCl_2$, $ZnCl_2$ and $CdCl_2$, and salts, such as $CaCl_2$ and KCl , were 5 mM, except 0.5% $NaCl$ and 50 nM Na_2SO_3 , which release SO_2 in the water. The rice cell culture was maintained with indicated stress for 1 week, and then filtered through Whatman paper (No. 1) for the separation of rice cells from medium extracts. The collected cells were resuspended with a minimum volume of fresh AA2 media for the measurement of

cellular isoperoxidase levels. The activities of secretory extracellular isoperoxidases were measured in the remaining medium extract.

Enzyme purification and gel electrophoresis

The crude enzyme preparation was loaded on a CM-cellulose cation-exchange column pre-equilibrated with 5 mM sodium phosphate buffer (pH 6.0). The column was washed with the same buffer until the absorbance of the eluant containing all anionic isoperoxidases at 280 nm became zero. Isoperoxidase RC3 was eluted with 50 mM sodium phosphate buffer after eluting RC21 and RC2 completely with 30 mM sodium phosphate buffer (pH 6.0). The fractions containing isoperoxidase RC3 were applied to a Sephacryl S-100 column, and separated from other proteins. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as described by Laemmli (1970).

Determinations of molecular weight, isoelectric point and glycosylation

The native molecular weight of isoperoxidase RC3 was estimated by gel filtration chromatography on Sephacryl S-100 column, and subunit molecular weight of the enzyme was determined by SDS-PAGE. The isoelectric point (pI) of RC3 was determined on the polyacrylamide gel plate containing ampholine carrier-ampholite (pH 3.5-pH 9.0). Glycosylation of the enzyme was determined with the Schiff's reagent as a visualizing reagent for carbohydrate portion (Grossman and Neville, 1971).

Results and Discussion

The changes in the activities of cellular isoperoxidases and extracellular isoperoxidases in suspension culture are now being widely used as the indication of tissue responses toward environmental stresses (Macek *et al.*, 1996; Lin and Kao, 2001). We investigated the effects of various environmental stresses such as heavy metals, salts and low (high) temperature on the secretion of peroxidase isozyme into the medium in rice (*Oryza sativa* cv. *Nak-Dong*) suspension culture. As shown in Table 1, $CaCl_2$ was the most

effective stimulator of peroxidase secretion into the medium. About 11-fold and 10-fold

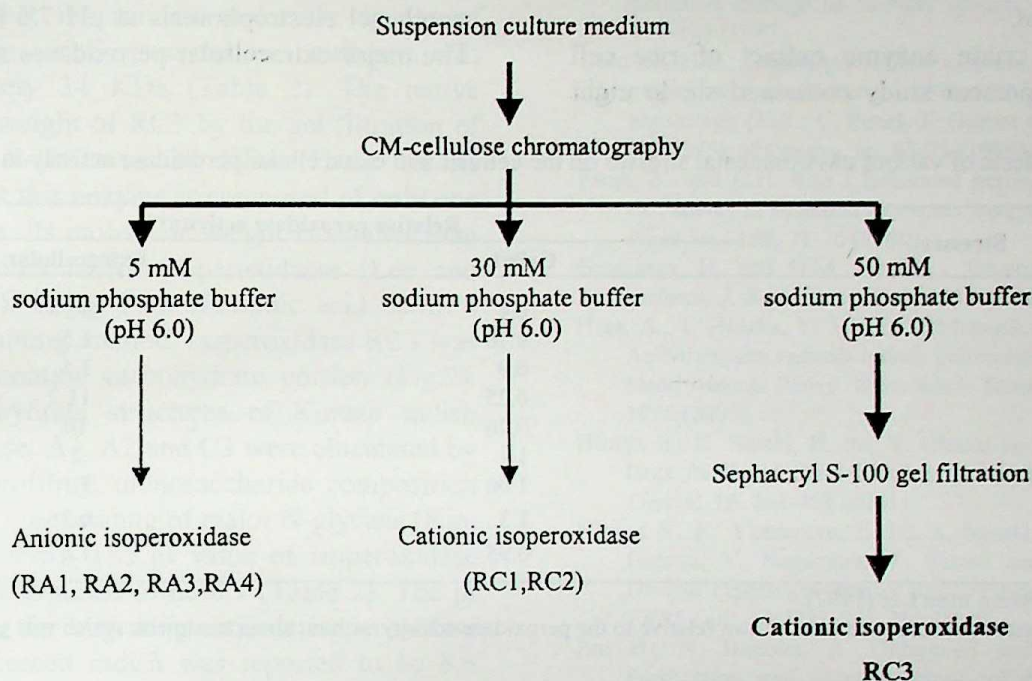


Fig. 1 : Scheme for chromatographic isolation of isoperoxidase RC3 from rice suspension culture.

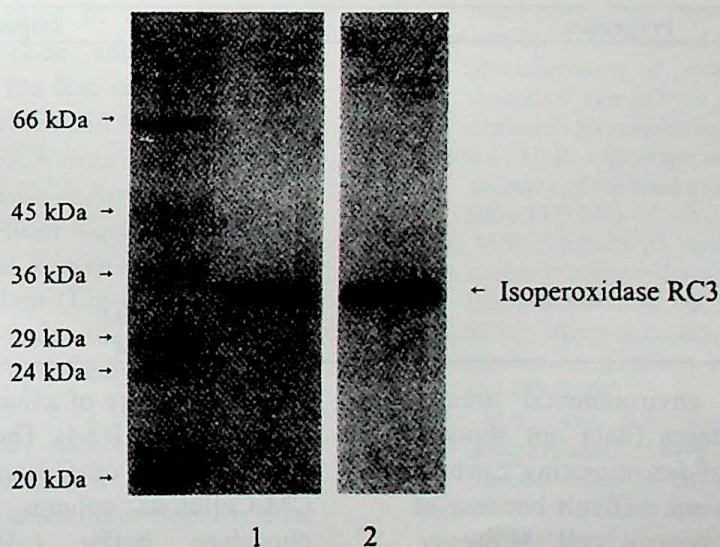


Fig. 2 : SDS polyacrylamide gel electrophoresis of isoperoxidase RC3 from rice suspension culture. The enzyme was stained with Coomassie brilliant blue R-250 for protein portion (lane 1) and Schiff's reagent for carbohydrate portion (lane 2). The molecular weight markers were as follows; bovine serum albumin, 66 kDa; chicken egg ovalbumin, 45 kDa; rabbit muscle glyceraldehyde-3-phosphate dehydrogenase, 36 kDa; bovine erythrocytes carbonic anhydrase, 29 kDa; bovine pancreas trypsinogen, 24 kDa; soybean trypsin inhibitor, 20 kDa.

enhancement of peroxidase secretion occurred with 5 mM CaCl_2 and 0.5% NaCl, respectively. On the contrary, notable reduction of the level of cellular as well as extracellular peroxidase

occurred with 5 mM ZnCl_2 . Na_2SO_3 , which is the soluble form of SO_2 , had no effect on the secretion of peroxidase into the medium. Cold stress of 4°C slightly reduced peroxidase

secretion, whereas heat stress of 40°C slightly enhanced secretory extracellular peroxidase level in the medium.

The crude enzyme extract of rice cell used in the present study contained six to eight

isoperoxidases designated as RC1, RC2, RC3, RA1, RA2, RA3 and RA4 when subjected to starch gel electrophoresis at pH 7.0 (Lee, 1997). The major extracellular peroxidases secreted into

Table – 1 : Effects of various environmental stresses on the cellular and extracellular peroxidase activity in rice.

Stresses	Relative peroxidase activity**	
	Cellular	Extracellular
ZnCl ₂	0.4	0.18
NiCl ₂	0.76	1.5
CdCl ₂	0.9	1.5
CaCl ₂	0.25	11.2
NaCl	0.26	10
KCl	1.1	2
Na ₂ SO ₃	1.06	1.1
4°C	1.3	0.75
40°C	0.95	1.6

* Data were supplied from Lee (1997).

** The values presented here have been shown relative to the peroxidase activity without stress treatment, which was given a value of 1.

Table – 2 : Physico-chemical properties of extracellular isoperoxidase RC3 from rice.

Properties	Isoperoxidase RC3
Molecular weight	
SDS-PAGE	34 kDa
Gel filtration	38 kDa
Glycosylation	Yes
Isoelectric point	8.9
Optimum pH	
Guaiacol	6.0
H ₂ O ₂	6.0
Km value	
Guaiacol	10.5 mM
H ₂ O ₂	3.2 mM

the medium by various environmental stresses were cationic isoperoxidases (data not shown). Among them, isolation of far migrating cationic isoperoxidase RC3 was very difficult because of its low content in the control cell. However, extracellular isoperoxidase RC3 could be easily purified after 5 mM CaCl₂ treatment due to the enhancement of RC3 secretion into the medium. Fig.1 shows the isolation scheme of isoperoxidase RC3 from rice suspension culture. Initial elution of CM-cellulose column with 5 mM sodium phosphate buffer (pH 6.0) after absorbing the column with crude enzyme preparation yielded

largely a mixture of anionic isoperoxidases (RA1, RA2, RA3 and RA4). The isoperoxidase RC3 was separated from other isoperoxidases by elution of CM-Cellulose column with 50 mM sodium phosphate buffer (pH 6.0) after eluting isoperoxidase RC1 and RC2 with 30 mM sodium phosphate buffer (pH 6.0). Sephacryl S-100 gel filtration of CM-cellulose fractions, which contain isoperoxidase RC3 activity, removed minor contaminating proteins and provided single polypeptide band in the SDS-polyacrylamide gel (Fig.2). Comparison of the relative electrophoretic mobility of the purified

isoperoxidase RC3 in the SDS-polyacrylamide gel with a set of proteins of known molecular weight indicated that the subunit molecular weight of RC3 from rice suspension culture was approximately 34 KDa (Table 2). The native molecular weight of RC3 by the gel filtration of Sephacryl S-100 was 38 KDa. These results suggest that this enzyme is composed of only one polypeptide. Its molecular weight is smaller than that of Korea radish isoperoxidases (Lee and Kim, 1994). Using PAS (Periodic acid Schiff's reagent) staining method, isoperoxidase RC3 was shown to contain carbohydrate portion (Fig.2). The carbohydrate structures of Korean radish isoperoxidase, A1, A2 and C3 were elucidated by N-glycan profiling, monosaccharide composition analysis and sequencing of major N-glycans (Kim and Kim, 1996). The pI value of isoperoxidase RC3 was determined to be 8.9 (Table 2). The pI value of the far migrating cationic isoperoxidase C3 from Korean radish was reported to be 8.6 (Lee and Kim, 1994). Isoperoxidase RC3 had pH optimum around 6.0 when guaiacol was used as a substrate, which is similar to that of Korean radish isoperoxidases (Lee and Kim, 1994). Isoperoxidase RC3 had the Km value of 10.5mM for guaiacol and 3.2 mM for H₂O₂ (Lee and Kim, 1994). Isoperoxidase RC3 was found to have higher Km values for H₂O₂ as compared to other cationic isoperoxidases from Korean radish, such as C1 and C3. H₂O₂ was known to be the major oxygen radical in the plant (Lin and Kao, 2001) and it increased greatly under the stress (Haga *et al.*, 2001). Therefore, it is unlikely that RC3 with low affinity for H₂O₂ may play the role of H₂O₂ detoxification. Detailed catalytic data and structural studies are needed to investigate the properties and roles of extracellular isoperoxidase RC3 in rice.

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The effects of early experience on subsequent feeding responses in the Tegu, *Tupinambis teguixin* (Squamata : Teiidae).

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Abstract : The purpose of this study was to assess the effects of early feeding experiences on subsequent responses to prey in the tegu, *Tupinambis teguixin*. Five-day old lizards were exposed to the odors of various prey and control substances on cotton-tipped applicators with the tongue-flick attack score (TFAS) chosen as the dependent variable. Each lizard was exposed to four stimuli : two controls (deionised water and cologne), and extracts from a mouse *Mus musculus*, and a lizard *Ameiva ameiva*, in a repeated measures, randomized block design, receiving one stimulus training session / day over a 40-day period. Tongue-flicks directed toward the applicator were counted over a 1 min period as well as the amount of time that elapsed from the first tongue flick to any bite that may have occurred. Live neonatal mice (but not *A. ameiva*), offered on a weekly basis, were used as a food source for tegus over a 10-month period. After 10 months, tegus were exposed to applicators containing control odors as well as those containing extracts from mice and lizards (*A. ameiva*). Mouse extracts elicited significantly higher TFAS as compared to those elicited by *A. ameiva* or control odors, suggesting that prey odors encountered in the environment shortly after hatching can influence prey preferences by these lizards later in life. These results also indicate that tegu lizards can learn to use specific odor cues associated with naturally occurring prey as releasers for subsequent hunting behaviors.

Key words : Early experience, Odor cues, Prey preferences, Tegu.

Introduction

Although in some species of snakes the recognition of, and responses to, natural prey species (or body extracts from these species) are innate (see Burghardt, 1977,1993; Burghardt *et al.*, 2000), the prey preferences of some ophidians can be altered by experience (Loop, 1970; Arnold, 1978; Stimac *et al.*, 1982; Lyman-Henley and Burghardt, 1995; Kats and Dill, 1998). Innate chemical discrimination of prey has also been reported for several lizards (Burghardt, 1973; Von Achen and Rakestraw, 1984; Garrett and Card, 1993; Cooper, 2000a), and actively foraging species that pursue their prey are known to utilize chemical cues to explore their habitat and detect the location of potential prey (Cooper, 1995; Cooper and Hartdegen 2000; Punzo and Madragon, 2002). An important and ecologically relevant question is to what extent food items encountered in the environment early in life affect preferences prey types as the lizards mature.

The teiid lizard *Tupinambis teguixin* (Latreille) is found in South America, from Venezuela to Uruguay (Ditmars, 1933; Ávila-Pires, 1995). Teiids and other scleroglossan lizards possess a well-developed olfactory apparatus and vomeronasal organs (Simon, 1983; Halpem, 1992; Cooper, 2000b), and are believed to hunt by utilizing both visual and olfactory cues to detect cryptic prey, or prey hidden beneath the ground or surface debris (Nobel and Kumpf, 1936; Bofill and Lewis, 1999; Punzo, 1990, 2001a,b). When a potential prey item is encountered, teiids exhibit tongue extrusions (tongue-flicking) in order to sample chemosensory cues and assess the suitability of the prey item (Cooper, 1990, 1995). Hatchlings and juveniles of *T. teguixin* feed on arthropods, small reptiles, and neonatal rodents and then switch to larger food items (eggs, birds, small mammals) as they mature (Ditmars, 1933; Bellairs, 1970; Fitzgerald *et al.*, 1991; Fitzgerald, 1994). The purpose of this study was to assess the effects (if any) of early feeding experiences on

subsequent responses to prey in the tegu, *Tupinambis teguixin* (prey recognition learning).

Materials and Methods

Nine hatchling tegus (6 males, 3 females) were used in these experiments. They were randomly selected from eggs deposited by 3 females that were bred in captivity and originally obtained from a commercial dealer in 1994. The eggs were kept in an incubator (precision Model 810, Boone, Iowa) at a temperature of $31^{\circ} \pm 1^{\circ}\text{C}$ and hatched on 5 Sept. 1998. Hatchling lizards were housed separately in reptile cages (Bush Herpetological Supply, Neodesha, Kansas, USA; Model S48T : 122 cm wide, 56 cm high, 53 cm deep). Each cage was provided with water in a glass bowl and ambient temperatures were maintained at $28 \pm 1^{\circ}\text{C}$ by Perlco heating elements (Bush Herpetological Supply, Model HEHW3). Each cage contained a plastic hide box where lizards could seek shelter. The floors of cages were provided with a commercial substrate consisting of a mixture of corncob shavings and sawdust, which was changed on a weekly basis.

Hatchling lizards were given a 4-day period to acclimate to their cages before any experiments were initiated. An experimental procedure similar to that described by Stimac *et al.* (1982) in their studies on prey recognition learning in red spitting cobras, *Naja mossambica pallida* was used with one notable exception. Stimac *et al.* (1982) exposed naive cobras to live prey sequestered in transparent Plexiglas cubes containing holes through which chemical cues could be detected, and measured the mean tongue-flicking rate (TFR) elicited by the live prey. In the present study, lizards were exposed to the odors of various prey and control substances on cotton-tipped applicators, and the dependent variable was the tongue-flick attack score, TFAS. This consists of a tongue-flick based index that takes into account not only extrusion of the tongue to the stimulus source but also biting (attack) of the applicator (Cooper and Burghardt, 1990).

Prey chemical stimuli were obtained by dipping the cotton tip of a wooden applicator into

deionised water, and then rubbing it over the lateral, dorsal and ventral body surfaces of the prey. Control animals were exposed to applicators containing deionised water or cologne (Mennen Skin Bracer, menthol) (Cooper and Vitt, 1989).

Experiments were initiated on the fifth day after hatching, and the hide boxes were removed 5 min before the start of each trial. To summarize the experimental procedure (test 1), each tegu was exposed to four stimuli (two controls, and extracts from a mouse and a lizard, *Ameiva ameiva*) in a repeated measures (randomized block) design (Stimac *et al.*, 1982). Each lizard received one stimulus session per day over a 40-day period (thus, every lizard was exposed to each stimulus condition 10 times). Control odors were obtained by dipping the cotton applicator into either deionised water (DW) or cologne (CL). Prey chemical cues were obtained from the body surfaces of a 3-day old neonatal mouse (*Mus musculus*, NM) or the prey lizard (*A. ameiva*, AA). *Ameiva ameiva* represents a naturally occurring prey species for *T. teguixin* (Avila-Pires, 1995).

All trials were conducted between 1300 - 1500 hr. Temperatures during testing were the same as the rearing temperatures mentioned above. Temperature was controlled because TFR in squamates has been shown to vary with temperature (Cooper and Vitt, 1986). In each trial, the applicator was placed directly in front of the subject, approximately 1 cm in front of its snout. Tongue-flicks directed toward the applicator were counted over a one-min period if the lizard did not bite the applicator. If it was bitten in < one min, tongue-flicks before the bite were recorded as well as the amount of time (latency) that elapsed from the first tongue flick to the bite (Cooper and Vitt, 1989). TFAS was determined according to the method described by Cooper and Burghardt (1990) and used as an index of overall response strength. All statistical procedures followed those described by Sokal and Rohlf (1995). Homogeneity of variance was assessed using Hartley's test. A single-factor, randomized blocks ANOVA was used to test for

Early experience and feeding responses.

significant treatment effects and multiple comparisons were determined via Newman-Keuls tests (two-tailed, $p < 0.05$).

For food, a live neonatal *M. musculus* (but not *A. ameiva*) was offered to each tegu lizard on a weekly basis over a 10-month period. By the end of a seven-week period, all tegus were eating mice within 24 hr after they were offered. As the tegus grew, they were offered mice ranging in age from 12 days -one month. After 10 months (test 2), the lizards were exposed to applicators containing the same controls (deionised water and cologne), as well as those containing the extracts from neonatal mice, furred

mice (PM, 12 -30 days old), and adults of *A. ameiva* (AA), utilizing the same procedures described above. *Ameiva ameiva* were obtained from a captive breeding population reared in laboratory since 1991.

Results and Discussion

The responses of *T. teguixin* are shown in Table 1. There were no significant differences between the responses of males and females ($P > 0.5$); thus, the data for both sexes were combined for analyses. The tegu tongue-flicked and / or bit

Table - 1 : Mean tongue-flick attack scores (TFAS) for juvenile (test 1) and 10-month old (test 2) tegus (*Tupinambis teguixin*) to various prey odor cues (NM = neonatal mice, *Mus musculus*; FM = furred mice; AA (the lizard, *Ameiva ameiva*) and control substances (CL = cologne; DW = deionised water) presented on cotton swabs. Data expressed as means (N=9) with standard errors (SE) and the range also listed. See text for details.

	Test 1				Test 2				
	DW	CL	AA	NM	DW	CL	AA	NM	FM
Mean	7.2	8.1	24.2	33.6	6.5	7.4	17.4	40.7	43.4
SE	1.1	1.5	3.3	7.6	0.9	1.2	2.6	9.1	7.8
Range	1-12	3-17	5-31	7-62	2-11	1-15	6-28	9-73	11-68

the applicators under all conditions. There was an overall significant effect of odor treatments on TFAS in test 1 ($F = 14.09$, $p < 0.001$) and test 2 ($F = 16.13$, $p < 0.001$) conditions. In the first test condition (juveniles), TFAS were much greater in response to odors from *M. musculus* as compared to *A. ameiva* ($P < 0.05$) or either control stimulus.

Similar results were obtained for 10-month old tegus (test 2), with the responses to prey odors associated with *A. ameiva* ($P < 0.01$) and *M. musculus* ($P < 0.01$) being greater than those for either control were. However, the responses to mouse odors were significantly greater than they were toward lizard odors ($P < 0.01$), and there was no difference between response rates toward NM vs. PM odors ($P > 0.5$). All variances were homogeneous ($F_{\max} = 2.14$, $p > 0.15$). The Newman-Keuls comparisons showed that the responses to lizard odors and mouse odors were significantly greater than for either

control substance ($P < 0.01$). The responses to deionised water and cologne did not differ under either test condition or between test conditions ($P > 0.10$). In addition, the responses to NM odors in test 2 were significantly greater than the initial responses to NM odor in test 1 ($t = 6.3$, $p < 0.05$).

Tupinambis teguixin is known to feed on a variety of lizards (including *A. ameiva*) and rodents under natural conditions (Ditmars, 1933; Bellairs, 1970; Ávila-Pires, 1995), and even shortly after hatching, they exhibited strong TF responses to odor cues from both lizards and mice during these tests. This is in general agreement with previous studies showing that responses of squamates to the odors of potential prey have some innate component (Burghardt, 1969, 1970, 1973, 1993; Yon Achen and Rekestraw, 1984; Graves *et al.*, 1987; Garrett and Card, 1993; Kaufman *et al.*, 1996; Cooper, 1997; Cooper and Hartdegen, 2000). Nonetheless, their increased

response rate to odors associated with mice (vs. *A. ameiva*) at 10 months of age, a prey item to which they had been repeatedly exposed to and had fed upon since five days after hatching, suggests that the increased sensitivity toward mouse odors may be based upon their recognition of specific chemical components of these rodents and that their early experience of actually feeding on mice was required for the development of this differential response later in life.

The exposure of a predator to a particular prey species early in life may increase its subsequent attentiveness toward chemical cues associated with that prey item. This type of preference formation has been referred to as prey recognition learning (Stimac *et al.*, 1982). However, because exposure to the chemical cues occurs early in life (presumably during a sensitive period), some form of olfactory imprinting might also be involved (Davey, 1989; Punzo and Kukoyi, 1997; Punzo, 2001a).

The results of this and similar experiments do not imply that *T. teguixin* will learn to accept any prey item simply based on an early period of exposure. However, they do suggest that squamates can learn to use specific odor cues associated with naturally occurring prey species as releasers for subsequent hunting behaviors.

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Dynamics of nitrogen, phosphorus, algal biomass, and suspended solids in an artificial lentic ecosystem and significant implications of regional hydrology on trophic status.

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Abstract : Chemical and biological parameters were analyzed to examine how regional hydrological fluctuations influence water quality of a artificial lentic ecosystem over a two-year period. The intensity of seasonal monsoon rain accounted for most of annual inflow and discharge and influenced flow pathway (interflow vs. overflow), resulting in a modification of chemical and biological conditions. Sharp contrasting interannual hydrology of intense vs. weak monsoon occurred during the study. The intense monsoon disrupted thermal stratification and resulted in ionic dilution, high TP and high inorganic solids (NVSS) in the headwater reach. The variation of NVSS accounted 75% of TP variation (slope = 4.14, $p < 0.01$, $n = 48$). Regression analysis of residual chlorophyll-a (Chl) versus flushing rate indicated that short hydraulic retention time and high mineral turbidity affected algal growth in the headwater reach during summer monsoon. In contrast, severe drought during weak monsoon produced strong thermal stratification, low inorganic solids, high total dissolved solids (TDS), and low TP in the entire system. In addition, Chl concentrations were controlled by phosphorus. Based on the physical, chemical and biological parameters, riverine conditions, dominated during the intense monsoon, but lacustrine conditions were evident during the weak monsoon. The interannual dynamics suggest that monsoon seasonality is considered the main forcing factor regulating overall functions and processes of the waterbody and this characteristic has an important implication to eutrophication of the system.

Key words : Nutrients, Lentic ecosystem, Algal biomass, Eutrophication, Seasonality.

Introduction

Eutrophication is a major cause of water quality deterioration in many Korean reservoirs. Recently, regional reservoirs have documented excessive anthropogenic nutrient enrichment (Kim *et al.*, 1985; Kim *et al.*, 1989; Ahn *et al.*, 1989), decreased transparency (Cho *et al.*, 1991), and frequent algal blooms (Kim *et al.* 1988; Cho *et al.* 1989). Eutrophication of these systems has mainly been attributed to organic feed used in in-lake fish farms (Choi *et al.*, 1988; Cho *et al.*, 1991) and agricultural runoff (Kim *et al.*, 1997). Studies of Taechung and Soyang reservoirs demonstrated that P input from the farms was estimated at > 45 percent of total P-loads (Cho *et al.*, 1991) and exceeded Vollenweider's (1976) dangerous loading level (Kim *et al.*, 1989).

Korean reservoirs potentially have unique limnological characteristics determined by summer Asian monsoon conditions during July - August. Large seasonal variations in water quality are expected in Korean reservoirs because one third of the annual total rainfall occurs during the monsoon period. Inflow during flood events can result in rapid flushing (Lind, 1993) and may have a dominant influence on ionic salinity, nutrient input, and algal biomass (Hoyer and Jones, 1983; Soballe and Bachmann, 1984; Ford, 1990), thereby modifying reservoir function. Studies of waterbodies influenced by monsoons in South Asia have demonstrated how flushing altered water chemistry, particularly salinity and nutrients (Singh, 1985; Banerjee *et al.*, 1983; Lohman *et al.*, 1988). Water quality, therefore, may be regulated by the duration and intensity of the monsoon in any

given year. Taechung Reservoir was constructed in 1980 and eutrophication has proceeded quickly since 1985 (Choi *et al.*, 1988). Studies of this reservoir have emphasized impacts of a wastewater disposal plant and fish farms on nutrients, algal biomass, and transparency (Choi *et al.*, 1988; Cho *et al.*, 1991; Choi and Lee, 1991). Despite recent work, little is known about how the unique monsoon climate and morpho-hydrodynamic characteristics influence chemical and biological processes in the reservoir. This paper demonstrates

how contrasting monsoon conditions influence chemical and biological processes within the system, and why an understanding of monsoon characteristics is essential to understanding reservoir limnology in this part of Asia.

Materials and Methods

Descriptions of study location and sample correction : Taechung Reservoir is located in the middle of South Korea (36°50'N, 127°50'E) and

Table – 1: Annual mean total nitrogen (TN, mg L⁻¹) measured at the mainstem sites in 1993 (n=11) and 1994 (n= 4). Sampling sites are arranged by order of location along the axis of the reservoir from the headwaters to the dam.

Mainstem sites	1993		1994	
	Annual mean	Range	Annual mean	Range
St. 1	1.67	1.24-2.46	1.43	0.72-2.25
St. 3	1.60	1.27-2.29	1.40	0.74-2.16
St. 4	1.58	1.17-2.33	1.46	0.86-2.11
St. 7	1.62	1.27-2.30	1.60	0.99-2.19
St. 8	1.72	1.27-2.43	1.64	1.05-2.52
St.10	1.58	1.19-2.27	1.57	0.95-2.11
St.14	1.53	1.27-1.96	1.40	1.09-2.07
St.15	1.57	1.35-2.12	1.35	1.01-2.12
St.16	1.51	1.20-1.90	1.30	1.08-1.96
Mean	1.60	1.17-2.46	1.46	0.72-2.52

Table – 2 : Regression analyses of TP ($\mu\text{g L}^{-1}$) against non-volatile suspended solids (NVSS, mg L⁻¹) in the headwater, mid-lake, and downlake zones in the high-flow year. The ANOVA test was used for the p-values.

Year	Zone	Sample # (n)	Slope	Intercept	p-value	R ²
1993	Headwater	48	4.14	29.9	< 0.001	0.74
	Mid-lake	60	5.20	20.9	< 0.01	0.48
	Downlake	96	-4.57	23.6	0.21	0.05
1994	Headwater	52	4.79	21.0	< 0.01	0.30
	Mid-lake	65	2.64	21.1	0.20	0.04
	Downlake	104	1.79	10.1	0.12	0.06

was formed in December 1980 by impounding the Keum River about 150 km upstream from its estuary. The selection of sampling sites in the Taechung Reservoir was based on the morphometry along the longitudinal axis and the position of external nutrient loads to the reservoir.

Along the main axis of the reservoir, we chose 9 mainstem sites (site 1, 3, 4, 7, 8, 10, 14, 15 and 16) and 8 embayment sites (site 2, 5, 6, 9, 11, 12, 13, and 17). In this study the headwater, middle, and downlake zone typically refer to sites 1 - 4, 5 - 9, and 10 - 17, respectively and the mean depth

at three zones was 8m, 16m and 28m, respectively. The distance from site 1 to site 17 (near the dam) is about 49km and site-to-site the distance interval is about 2.9 km. Surface water samples were collected from these 17 sites twice each month from April 1993 to November 1994 (except in winter, January - February). Herein, we use the terms "premonsoon" for the period of January to June, "monsoon" for the period of July to August, and "postmonsoon" for the period of September to December in describing temporal conditions.

Analytical methods : Water samples were covered to prevent exposure to direct sunlight, stored in ice, and either preserved or analyzed in the laboratory within 12 - 36 hours. Secchi transparency (20 cm disk), water temperature and dissolved oxygen (YSI Model 51B meter) were measured at the time of sample collection. Specific conductance (YSI Model 33) was measured in the laboratory. Total nitrogen (TN) was measured by the second derivative method after a persulfate digestion (Crompton *et al.*, 1992). Total phosphorus (TP) was determined using the ascorbic acid method after persulfate oxidation (Prepas and Rigler, 1982). Major anions such as sulfate (barium turbidimetric method), chloride (mercuric nitrate titration), and bicarbonate were determined using standard methods (APHA, 1985). Cations including calcium, magnesium, sodium, and potassium were determined on acid-preserved samples by atomic absorption spectrophotometry (Varian AA-20, APHA, 1985). Total dissolved solids were estimated from the sum of cations and anions. Total suspended solids (TSS) were determined by filtering water through preweighed Whatman GF/C filters. Filters were weighted after drying at 103°C for 1 hour. Non-volatile suspended solids (NVSS) were determined by combustion at 550°C for 1 hour (APHA, 1985) and volatile suspended solids (VSS) were determined by differences with appropriate corrections made for blanks. Chlorophyll-*a* (Chl) concentration was measured by using a spectrophotometer (Bechman Model DU - 65) after extraction in hot ethanol (Sartory and Grobbelaar, 1984). Nutrient analyses were

performed in triplicate; suspended solids and Chl were measured in duplicate.

Results and Discussion

Seasonal hydrology showed sharp contrasts between the monsoons of 1993 and 1994 (Fig. 1). Total precipitation during the 1993 monsoon was 660 mm, which comprised 43 % of the total annual precipitation, but during the 1994 monsoon, it was only 251 mm (Fig. 1a). The distributions of rainfall resulted in an interannual variation of inflow volume (Fig. 1b) and discharge volume from the dam (Fig. 1c). Total inflow in 1993 was four times that of 1994 ($0.83 \times 10^9 \text{ m}^3$), and the summer inflow in 1993 was eight times greater than the summer of 1994 (Fig. 1b), indicating a high-flow year in 1993 vs. low-flow year in 1994. Seasonal changes of total outflow showed a similar pattern with inflows (Fig. 1d) and influenced the water stage. Water stage abruptly increased after the maximum inflow in July, peaked during the July-August period of the high flow year, and then continued to decline by December of the low-flow year (Fig. 1d).

Physical processes have major implications for controlling eutrophication in reservoirs (Vincent *et al.*, 1991; Lind *et al.*, 1993). A dominant process is the influence of density currents on the thermal stratification and mixing regime (Ford, 1990). The influence of density current on thermal structure was evident in the Taechung Reservoir. During the summer of the high-flow year, river water plunged to mid-lake (location 27 - 37 km) and traversed as a subsurface interflow (Fig. 2a). The interflow disrupted the thermal stratification and produced a metalimnetic warming greater than 4°C downlake, thereby increasing the thermocline depth by greater than 20 m. The inflow pattern directly influenced the timing of the fall overturn; because of metalimnetic warming, the overturn in the high-flow year occurred about 30 days earlier relative to that of the low flow year. The interflow also decreased the volume and thickness of the summer hypolimnetic hypoxia because of rapid

hypolimnetic discharge from the dam and the replacement of hypolimnetic volume by interflows.

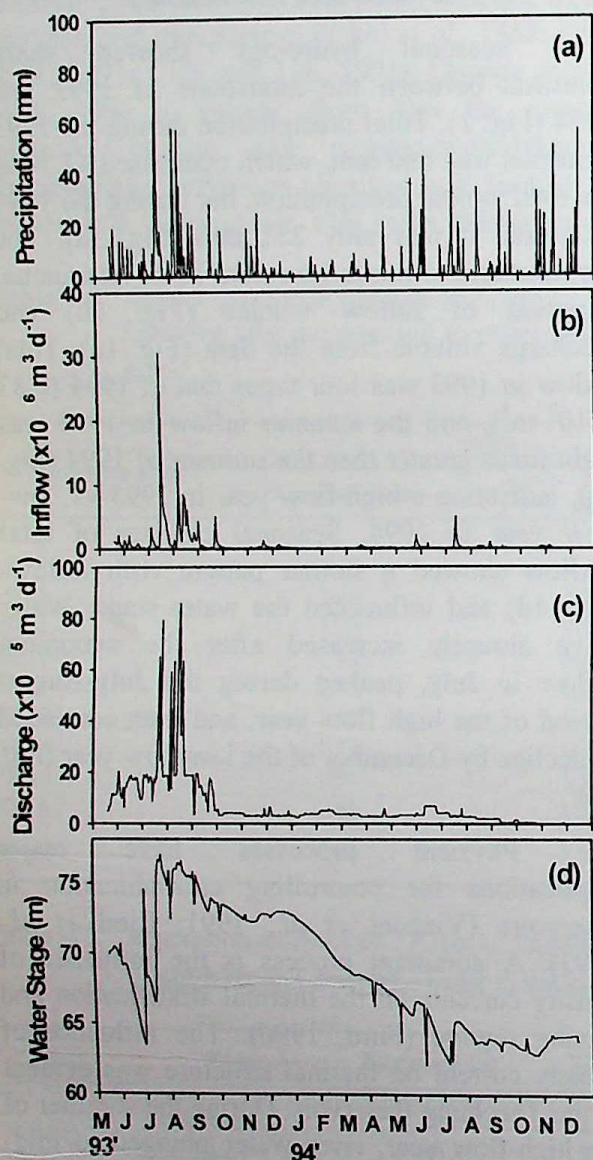


Fig. 1 : Hydrological changes over the two year period of 1993-1994.

For this reason, hypoxic volume in the high-flow year was less than in the low-flow year. In the low-flow year, strong thermal stratification found during the monsoon of the low-flow year, resulting in meta-hypolimnetic hypoxia. Severe anoxia in the low-flow year monsoon caused massive fish kills (i.e. *Hypomesus olidus*) in the middle reach of the reservoir.

a) Distance from the dam

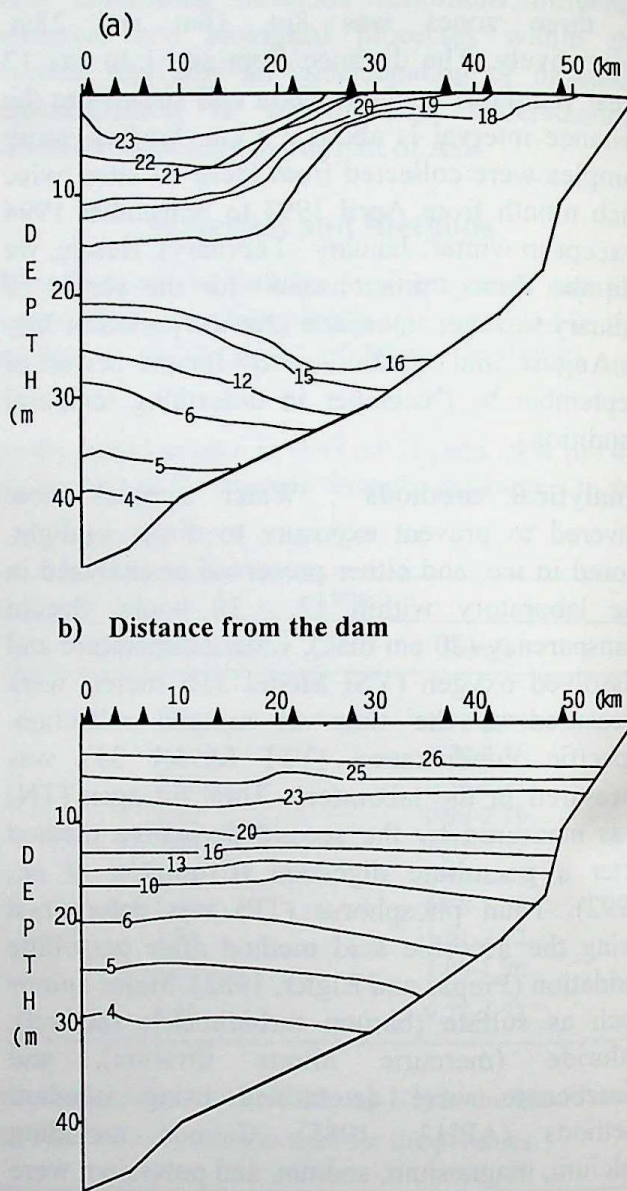


Fig. 2 : Isothermal pattern along the mainstem sites (triangle) from the headwaters to the dam in the monsoon of high-flow year (2 July: a) and monsoon of low-flow year (7 July: b).

The major mechanism regulating total dissolved solids (TDS), a measure of ionic salinity, in this system was dilution by monsoon inflow. Concentrations of TDS varied seasonally in response to the magnitude of the monsoon inflow (Fig. 3); TDS was lowest (42.2 mg L^{-1}) during the peak inflow in 1993 and highest (91.2 mg L^{-1}) during a drought period (monsoon) in 1994. Mean TDS values were below 70 mg L^{-1} in three seasons of high-flow year vs. above 70 mg L^{-1} in the three seasons of the low-flow year (Fig.

3A). In addition, there were no significant differences (p values > 0.8 , $n=16$) among the three seasons of the low-flow year. Mean TDS during the monsoon period

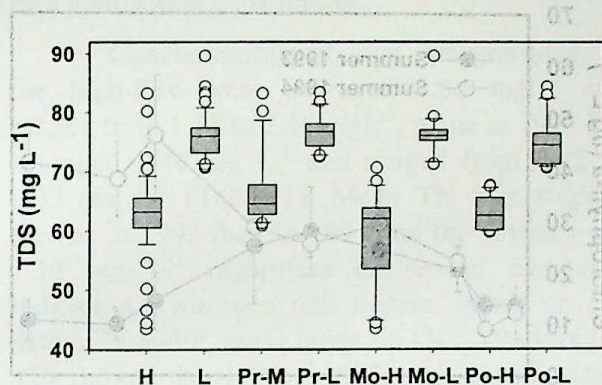


Fig. 3 : Seasonal and interannual changes of total dissolved solids (TDS, mg L^{-1}) in high-flow year (H) and low-flow year (L). In the figure, Pr-H, Mo-H, Po-H and Pr-L, Mo-L, Po-L indicate the premonsoon, monsoon, and postmonsoon in the high-flow year (1993) and low-flow year (1994), respectively.

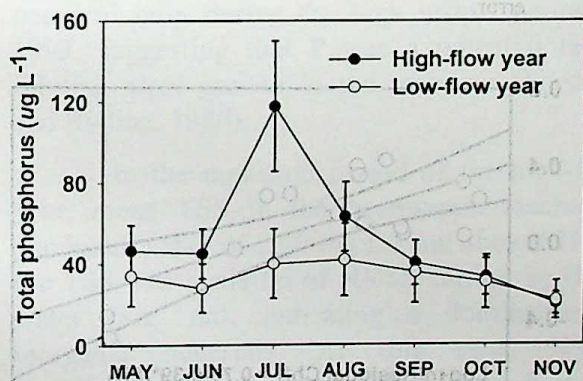


Fig. 4 : Monthly distribution of mean total phosphorus (TP) in the high-flow year and low-flow year.

of the high-flow year was significantly ($p < 0.05$, $n=16$) lower than TDS values of other seasons of both years. These declines of TDS were mainly attributed to decreases in calcium and bicarbonate concentrations among all cations and anions.

Annual mean P-budget was regulated mainly by the intensity of the summer monsoon. In-lake TP, based on the seasonal average of May-November, was significantly ($p < 0.001$) greater in the high flow year (mean = $38 \mu\text{g L}^{-1}$; range = $6 - 197 \mu\text{g L}^{-1}$) than in the low-flow year

(mean = $25 \mu\text{g L}^{-1}$; range = $6 - 77 \mu\text{g L}^{-1}$; Fig. 4). Thus, the trophic state based on the TP criteria of Nurnberg

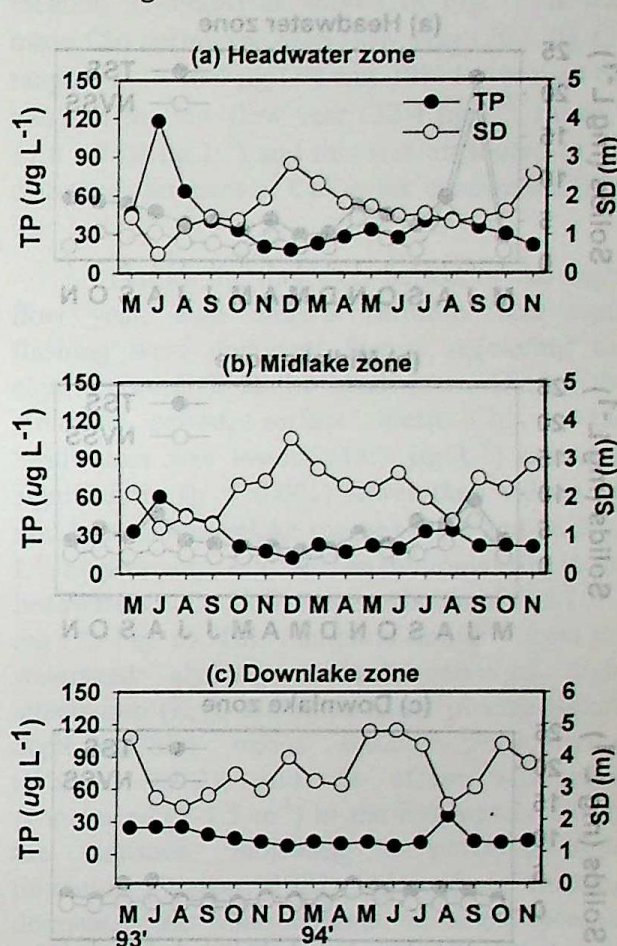


Fig. 5 : Seasonal fluctuations of TP and Secchi depth (SD) in the headwater, midlake, and downlake zones in both years.

(1996) was greater in the high-flow year (eutrophic) than in the low flow year (mesotrophic). The marked difference between the two annual means was attributed to summer P-input during July through August; the summer mean in the high-flow year was greater by at least $50 \mu\text{g L}^{-1}$ than the value in the summer of the low-flow year (Fig. 4).

Concentrations of TP in the headwaters were directly influenced by the hydrograph within the watershed (Fig. 5, Fig. 1b). In early monsoon period of the high flow year (July 1, 1993), TP in

the headwaters increased by three fold relative to the premonsoon period (Fig. 5a).

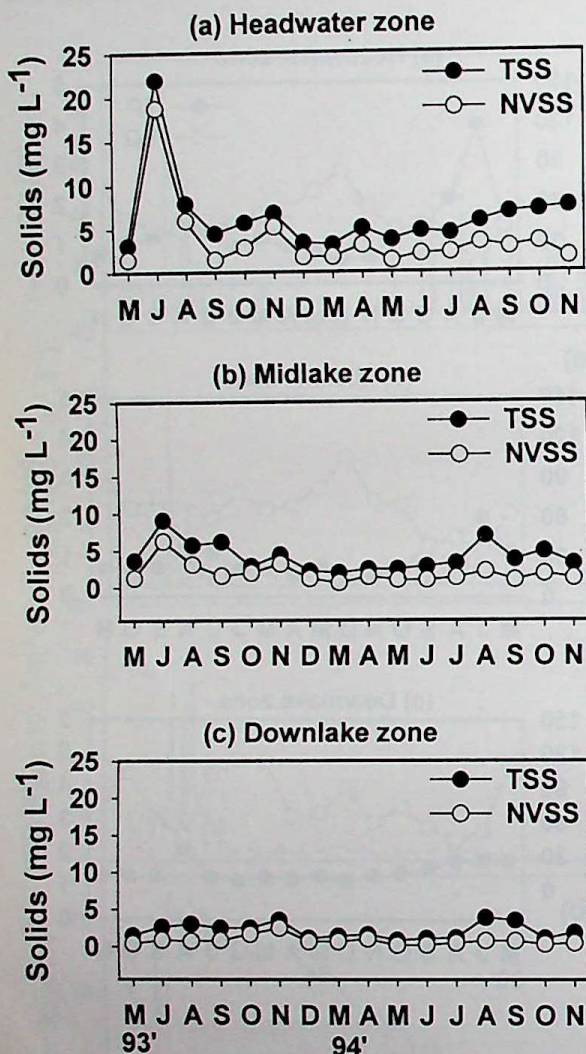


Fig. 6 : Seasonal fluctuations of total suspended solids (TSS) and non-volatile suspended solids (NVSS) in the headwater, midlake, and downlake zones in both years.

Some 74% in the variation of TP in the headwaters was accounted for (slope = 4.14, $p < 0.001$, $n = 48$) by the variation of non-volatile suspended solids (NVSS) as shown in Table 2. Values of TP, however, decreased to less than 60% during the September - October of high-flow year. The temporal variation in low-flow low compared to the high-flow year; monthly mean TP in the headwaters and mid-lake ranged from $22\text{--}41\mu\text{g L}^{-1}$ and $19\text{--}34\mu\text{g L}^{-1}$, respectively (Fig. 5a,b) and their variations ($R^2 = 0.30$ in the

headwaters; $R^2 = 0.04$ in the midlake) were marginally explained by the NVSS (Table 2). The two maximum values in both

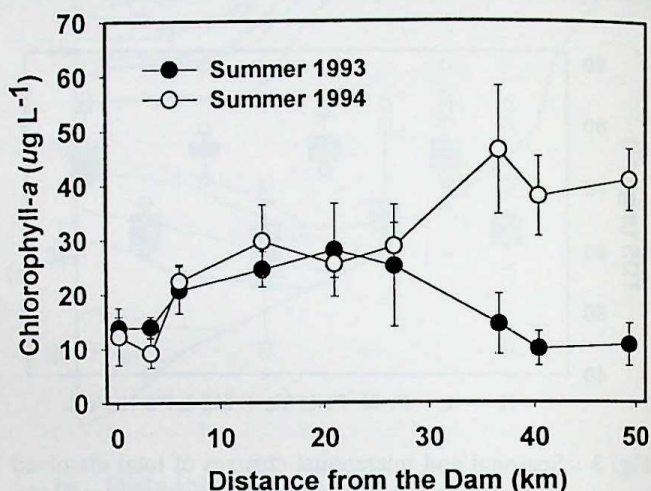


Fig. 7 : Longitudinal distribution of chlorophyll-a during summer 1993 and 1994. Each data point in 1993 and 1994 indicates a mean value by site during monsoon, and the vertical bar indicates a standard error.

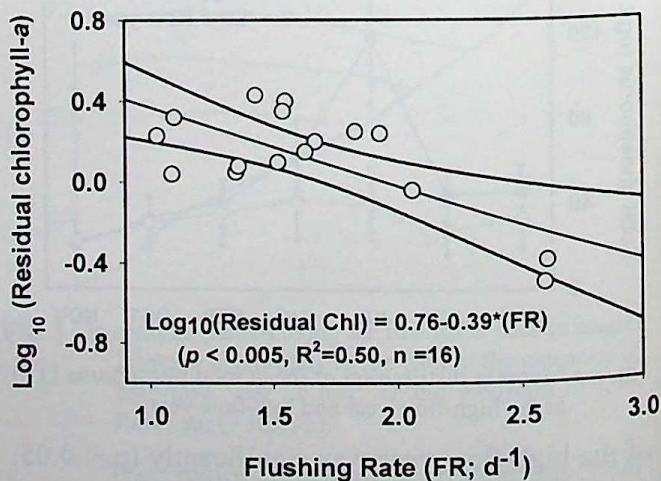


Fig. 8 : Regression analysis of residual chlorophyll-a [measured log-transformed Chl minus predicted log-transformed Chl based on empirical model of Jones and Bachmann (1976)] against monthly mean flushing rate.

zones were 65% and 43% lower, respectively, than in those two zones in the high-flow year. Variation of TP, however, was minimal at the downlake region (Fig. 5c) and exhibited no

relation ($R^2 < 0.07$) with NVSS in the downlake region in both years (Table 2). The marked interannual variability in the headwaters was a result of the difference in external P-input caused by a contrasting flow regime.

Concentrations of total nitrogen (TN) in the high-flow year averaged 1.60 mg L^{-1} and ranged from 1.17 to 2.46 mg L^{-1} , while in 1994 TN averaged 1.46 mg L^{-1} and ranged from 0.72 to 2.52 mg L^{-1} (Table 1). Mean TN was slightly greater in 1993 than in 1994 and the values were 1.30 mg L^{-1} regardless of season and site, indicating a nitrogen rich system. Based on our data of N and P, mass ratios of TN : TP averaged 120 during the 1993 - 1994 period and varied from 10 to 226. The mean ratio in 1993 was 63 and in 1994, it was 179, indicating a large temporal variation. This temporal variation was due to a fluctuation of P rather than changes of N. During the study about 97% of the total observations ($n = 509$) of TN : TP was greater than 17. The remaining 3% of ratios less than 17 occurred only during the high inflow period of 1993, suggesting that P was a potential factor limiting algal growth in the reservoir (Forsberg and Ryding, 1980).

In the monsoon period of the high-flow year, mean TSS in the headwaters reached a maximum value of 20.0 mg L^{-1} and about 81% of the TSS was made up of NVSS carried by flood water (Fig. 6a), indicating a dominance of inorganic materials. At this time, NVSS contributed to the input of TP. It is based on the regression analysis of TP against NVSS (Table 2). In contrast, NVSS in low-flow year never exceeded values greater than 4 mg L^{-1} and VSS, measured as TSS minus NVSS, was dominant during all the seasons (Fig. 6), suggesting a larger contribution of algae or organic matter to total solids. Despite the dominance of the NVSS in the headwaters, NVSS varied little at the downlake region (Fig. 5c).

During the study chlorophyll-*a* (Chl) averaged $20 \mu\text{g L}^{-1}$ and varied from $1.7 \mu\text{g L}^{-1}$ to $172.7 \mu\text{g L}^{-1}$. Mean Chl ($21.8 \mu\text{g L}^{-1}$) in the high-flow year was similar to the value in the low-flow

year ($22.7 \mu\text{g L}^{-1}$), but a distinct difference in the annual mean Chl (by site) between the two years, however, occurred in the headwaters (sites 1 - 4; location 37-49km) as shown in Fig. 7. In-lake mean Chl during the high-flow year ($20.1 \mu\text{g L}^{-1}$, range = $2.2 - 40.4 \mu\text{g L}^{-1}$) was 38% lower than the mean of the low-flow year ($32.4 \mu\text{g L}^{-1}$, range = $11.8 - 74.8 \mu\text{g L}^{-1}$) and this was attributed to the distinct differences of Chl in the headwaters (Fig. 7).

During the monsoon period of the high-flow year, high mineral turbidity and rapid flushing were dominant factors regulating the algal chlorophyll in the headwaters. During the monsoon period, surface mean Chl in the headwaters was lowest ($11.7 \mu\text{g L}^{-1}$) and was significantly ($p < 0.001$) lower than values for mid-lake and downlake reaches (26.6 and $18.3 \mu\text{g L}^{-1}$, respectively; Fig. 7). The minimum Chl in the headwaters coincided with maximum NVSS (17.4 mg L^{-1} , Fig. 6). This inorganic turbidity from the watershed abruptly caused non-algal light attenuation (K_{na}), estimated as an inverse Secchi depth ($1/\text{SD}$) minus $0.025 \times \text{chlorophyll-}a$ (Walker, 1982), and its effect was most pronounced ($> 1.5 \text{ m}^{-1}$) in the headwaters during the monsoon, indicating a potential light limitation (Walker, 1982). Also, rapid flushing dominated the water column, so algal biomass might have been affected by the short hydraulic retention time (HRT). In fact mean HRT measured during the peak-flow was 2.2 d in the headwaters vs. 32 d in the downlake reaches. Thus, values of Chl (range = $10.7 - 28.0 \mu\text{g L}^{-1}$) in the headwaters decreased with an increase of flushing rate (range = $32 - 121 \text{ yr}^{-1}$) and were strongly correlated ($r = 0.90$; $p < 0.05$) to HRT values. Fig. 8 shows the regression analysis for flushing rate against residual Chl values [Log-observed Chl minus Log-predicted Chl calculated using the TP-Chl empirical model of Jones and Bachmann (1976)]. The negative values of residual Chl during the monsoon were observed in the high flushing range (Fig. 8), indicating a potential washout of algal biomass. Similar results are found in non-monsoon lakes where HRT is

below 10 d (Hoyer and Jones, 1983; Soballe and Bachmann, 1984; Lind *et al.*, 1993). In addition, during the monsoon period of the high-flow year, particulate P accounted for greater than 60 % of TP, and inorganic solids (NVSS) were compared greater than 90% of the TSS. This result suggests that ambient nutrient content was sufficient ($TP > 100 \mu\text{g L}^{-1}$; $TN > 1.3 \text{ mg L}^{-1}$) but bio-available P decreased due to phosphorus adsorption reactions with suspended particles (Cowen and Lee, 1976).

In contrast, phosphorus was the major determinant regulating algal biomass during all the seasons of the low-flow year. Monthly mean Chl was less than $20 \mu\text{g L}^{-1}$ in spring (March - June) and peaked to $42.2 \mu\text{g L}^{-1}$ in the mid monsoon. The maximum was 2.3 times greater than the mean in the spring. During the massive algal blooms ($> 40 \mu\text{g L}^{-1}$ Chl; Maceina, 1993; Havens *et al.*, 1995) in the mid-monsoon period, Chl : TP ratios were greater than 1.0, indicating high Chl yields at a given P (Walker, 1982; Knowlton and Jones, 1995). During the monsoon, NVSS values were less than 6 mg L^{-1} , indicating a low flushing rate and mineral turbidity. Thus, non-algal light attenuation (K_{na}) never exceeded 0.5 m^{-1} at all sites during the 1994 monsoon, indicating that light availability was enough for algal growth (Walker, 1982). Under such circumstances, the key factor controlling algal growth was ambient P concentration.

It is evident that under the low-flow condition, the primary component influencing levels of algal production was phosphorus. Under high flow-conditions, algal Chl was affected by various factors and showed high spatial heterogeneities within the reservoir due to partial differences of HRT at each site. One of the most important features in this P-limited reservoir was little increase of in-lake productivity during the monsoon of the high-flow year in spite of major inputs of phosphorus from the watershed. External P supply during the monsoon of the high-flow year was over twice that of the monsoon of low-flow year. Overall in-lake mean Chl during the monsoon of the high-flow year, however, was significantly ($p < 0.01$) lower than

that during monsoon of low-flow year ($28.1 \mu\text{g L}^{-1}$, Fig. 8). Less biomass in the high-flow year was attributed mainly to decreases in the mainstem headwater-sites. Also, ratios of overall in-lake mean Chl to TP-input from the watershed were minimal ($\text{Chl} : \text{TP} < 0.08$) during the monsoon of the high-flow year. This phenomenon was also explained by interflow current (Lind *et al.*, 1993) as well as several factors mentioned above. During the 1993 monsoon, river water entered the reservoir as an interflow (Fig. 1) and the water mass moved in the stratum below the photic zone (approximately, $2.3 \times \text{Secchi depth}$; 1.4 m in the headwaters to 8.2 m downlake). As shown in previous studies of non-monsoon regions (Ford, 1990; Soballe *et al.*, 1992; Kennedy and Walker, 1990; Cooke *et al.*, 1993; Knowlton and Jones, 1995), nutrient-rich water was isolated from the trophogenic epilimnion within the midlake and downlake reaches. This is due to a density difference between the reservoir and inflow water, resulting in little contribution to the reservoir productivity (Ford, 1990; Soballe *et al.*, 1992) and eutrophication. These findings indicate important implications for lake management in this monsoon-reservoir. We believe that this system, influenced by the Asian monsoon, seems to differ in its function and processes compared to lakes of non-monsoon regions. In the lakes of North America, summer algal Chl is frequently determined by spring or summer TP (Dillon and Rigler, 1974; Walker, 1982), in-lake nutrient concentrations are closely correlated to input of nutrients from the watershed and determine inlake algal Chl concentrations. For these reasons, empirical models using summer (or spring) TP-summer Chl relation have been widely used for lake management in North America and Europe and have well predicted lake eutrophication. This management tool, however, may be ineffective in the Southeast Asian regions where cyclic seasonal monsoons occur and intense monsoon rain occurs in a short period (i.e., like July-August in this region). This reason is why the monsoon acts a "pulse effect or deterministic instability" (Straskraba *et al.*, 1993) to waterbodies of Asian

regions. This hypothesis needs to be tested using long-term regional data in the future.

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Darkened *Xenopus* tadpoles appeared with neurochemical agents.

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Abstract : Skin darkened tadpoles sometimes appear spontaneously. Darkened was artificially induced in *Xenopus* larvae by yohimbine or chlorpromazine. These phenomena look like that are seen at pinealectomized or hypothalamus separated *Xenopus* larva. In this experiment, such a morphological color changed *Xenopus* larva is suggested by cause of inhibition of $\alpha 2$ -adrenargic receptor or dopamine receptor from gastrula stages.

Key words : *Xenopus laevis*, Melanin, Pigment cell, Neurochemicals, Morphological color change.

Introduction

The African frog *Xenopus laevis* larvae are sometimes found to have melanosome dispersed darkened individuals among the normal melanosome contracted lighter coloured ones. The reason why these natural morphological colour changes occur is unknown. In controlled conditions, pinealectomized or hypothalamus separated larvae become skin darkened, and under these circumstances, the reason is speculated to be the neural or hormonal control of the melanophores (Bagnara, 1960, 1963; Phlemann, 1976). However, numerous compounds may affect the state of the chromatophores (Katayama *et al.*, 1990; Morishita, 1987). In this experiment, we checked for morphological colour changes caused by a variety of compounds, and finally found that melanin pigment dispersion took place in the *Xenopus laevis* larvae in the presence of yohimbine or chlorpromazine.

Materials and Methods

Chemicals : All chemicals used were as follows: Dibutyl cyclic AMP (Sigma), 8-Br-cyclic AMP (Sigma), α -MSH (Sigma), yohimbine hydrochloride (Wako), chlorpromazine (Wako), phentolamine hydrochloride (Sigma), prazosine

hydrochloride (Sigma), (\pm) -sulpiride (RBI), haloperidol hydrochloride (Sigma).

Animals : Fertilized eggs of *Xenopus laevis* were obtained by the administration of HCG (Denka Pharmaceuticals, Kawasaki, Japan). Embryos were dejellied with 5.4% cystein-HCl (pH 7.45), then transferred into 100% Steinberg's solution at 20°C. The embryos were staged according to Nieukoop and Faber (1967).

Enzyme assay and melanin contents : Tyrosinase activity was measured in a solution containing 1mM dopa, 35mM sodium phosphate buffer at pH 6.8 and enzyme, in a total volume of 3ml at 35°C. One unit of enzyme activity was defined as that amount which produced 1 μ mol of dopachrome/ml/min (Koga *et al.*, 1992). Protein concentration was estimated by the method of Lowry *et al.* (1951).

Melanin contents of *Xenopus* larvae were measured by ESR spectra with slightly modification (Nebert *et al.*, 1963). ESR spectra were observed with JEOL X-band spectrometer at 100KHz field at 20°C. A magnetic field of 3300G, a microwave frequency of about 9.4 GHz, a microwave power of 5mW and a modulation width of 0.63G were adopted.

Results and Discussion

Fig. 1 and Fig. 2A, 2B, 2C show spontaneously appearing darkened larvae among usual colored larvae in natural conditions (Fig. 2D, 2G). The eyes of some larvae show some malformation (Fig. 2A, 2B arrow head). The appearances of such slightly enlarged black larva is not infrequent, as we can see at least a few darkened larvae from each female *Xenopus* delivery. The pinealectomized larvae show no melanin pigment contraction. Such maldeveloped larvae look like pinealectomized *Xenopus* larva after 60 minutes in the dark (Bagnara, 1960). Fig. 2D, 2G show normal *Xenopus* larva (stage 46) under normal circumstances. Yohimbine ($1.0 \times 10^{-5} M$) or chlorpromazine ($1.0 \times 10^{-6} M$) influence the *Xenopus* embryos from the gastrula stage, and after hatching the larvae begin to darken. Fig. 2E, 2H and Fig. 2F, 2I show skin darkened larvae administrated with these agents. Fig. 2G, 2H, 2I show low magnification of larvae on a white

background. After hatching the larvae were treated with the agents, the toxicity being increased until it was at the same concentration as at the gastrula stage, at which point the larvae were dead. Morphological color change was induced by yohimbine or chlorpromazine.

Fig. 3A shows the melanin content of *Xenopus* larvae treated with yohimbine or chlorpromazine. The total amount of melanin in the control larvae is higher than in the yohimbine or chlorpromazine treated larvae 1 to 2 days after hatching. At several days after hatching the melanin content of the agent treated larvae is higher than in the control. Tyrosine activity is shown in Fig. 3B. Normal larvae's tyrosinase activity is highest at the earlier part of its development. The enzyme activities of yohimbine treated larvae, however, increase during its development. In the case of chlorpromazine, there is a peak around 5 days after hatching. Normal tyrosinase activity is lower than the treated larvae's activity.

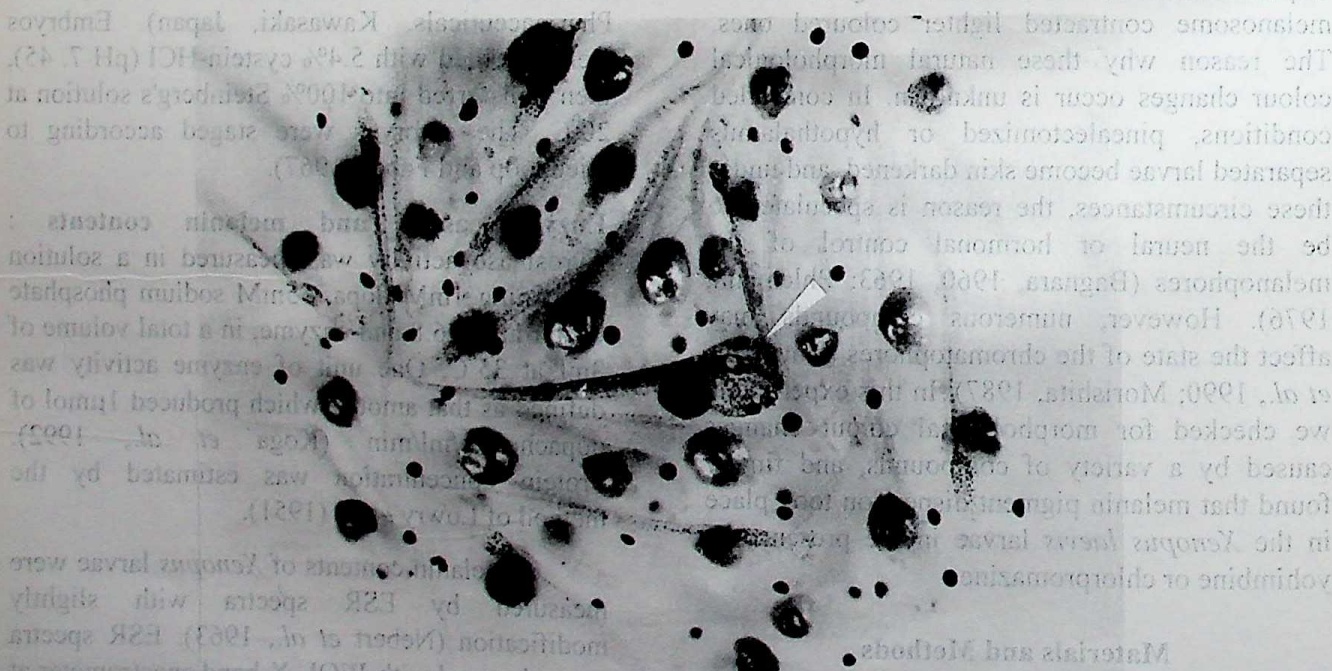


Fig. 1 : Darkened *Xenopus laevis* larva (arrow head) among normal larvae from one female *Xenopus* delivery.

From these results, we demonstrate morphological color changes of *Xenopus laevis* larvae with neurochemical agents.

In the present experiment, *Xenopus* larvae were darkened by being treated with yohimbine or chlorpromazine from the gastrula stages. These larvae look like the naturally occurring darkened

Darkened Xenopus tadpoles appeared with neurochemical agents.

Xenopus larvae among normal larvae. *Xenopus* larva with separation of the hypothalamus from the hypophysis by section of the prosencephalon also show melanophore expanded darkened larvae (Phlemann, 1976). The reason why darkened larvae occur is speculated to be the neural or

hormonal control of melanophore. We tested a series of such kinds of neural or hormonal agents. In this experiment, we focused on pigment dispersion agents or inhibitory compounds of pigment aggregation.

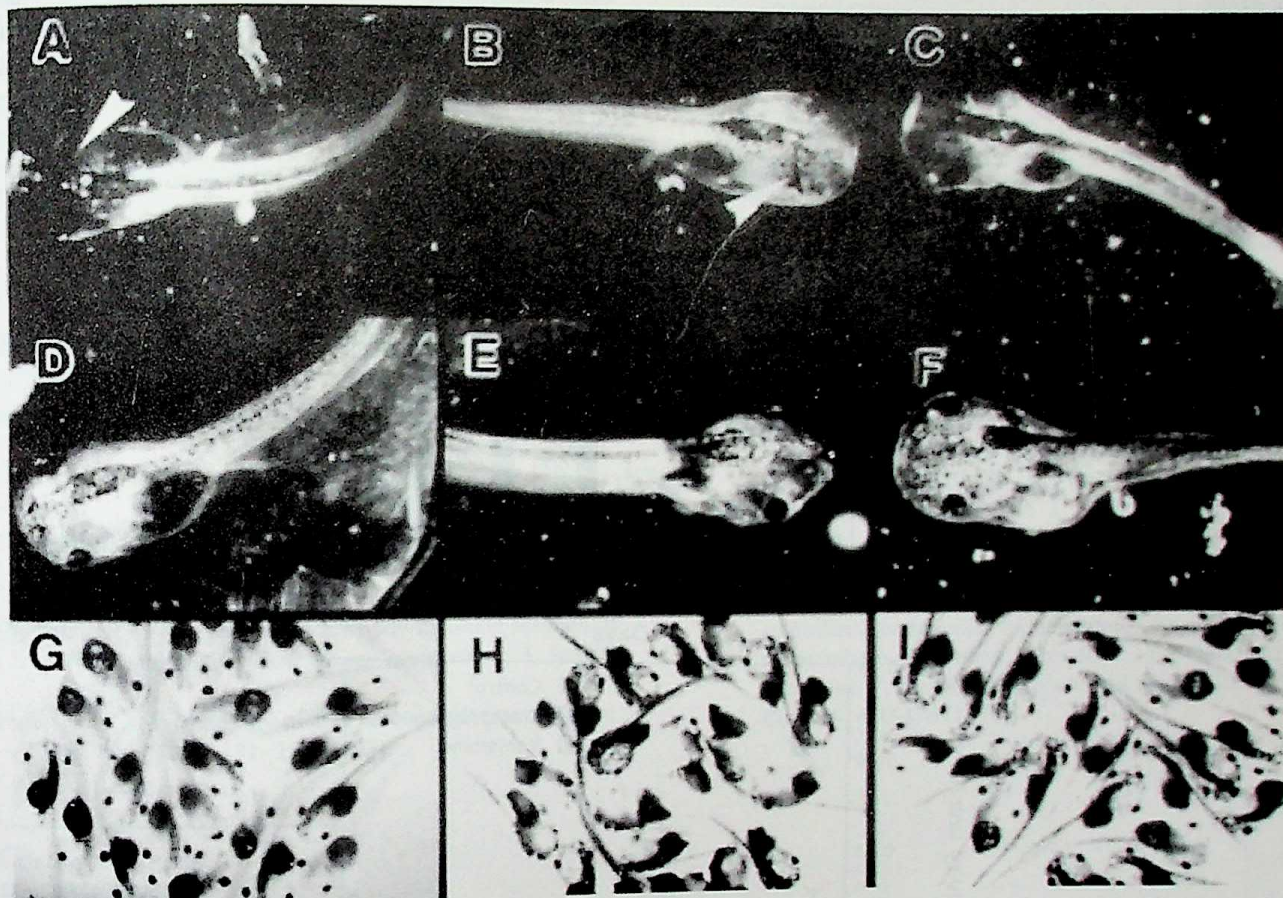


Fig. 2 : Maldevelopmental *Xenopus* larvae (stage 46) in normal control larvae. (A, B, C) maldevelopmental skin darkened larvae; (D, G) normal control; (E, H) and (F, I) treated with Yohimbine 10^{-5} M, and Chlorpromazine 10^{-6} M from the gastrula stage, respectively.

The most well known activity of the melanophore-stimulating hormone (MSH) is stimulation of the dispersion of melanin pigment (Shizume *et al.* 1954; Lerner, 1959; Lowry *et al.*, 1970). In amphibians, MSH does not play a role in early embryonic melanophore development (Wahn *et al.*, 1976). In our experiment, we got the same result. The cyclic AMP induces a discernible dispersion of pigment in fish melanophores (Fujii, 1993). In our experiment, treatment of cyclic AMP analogue (Dibutyryl

cyclic AMP) at the embryonic stages could not cause the dispersion of the melanin pigment.

Next, inhibiting agents for pigment aggregation were tested and we assumed alpha adrenargic antagonists or some kinds of blockers. Of the many $\alpha 2$ -adrenergic antagonists or D2-antagonists, only yohimbine or chlorpromazine was found to be potent melanophore-dispersion agents. Other $\alpha 2$ -antagonists (Phentolamine hydrochloride, Prazosine hydrochloride) and D2-antagonists ((RS)-(\pm)-Sulpiride, Haloperidol hydrochloride) show either toxic or no effect on

the melanin pigment dispersion at the same concentration range as the former agents ($1.0 \times 10^{-5} \text{M} \sim 1.0 \times 10^{-6} \text{M}$). We cannot give a good explanation for these results as many agents exert almost the same physiological functions, but seldom have the same effects on developmental processes.

Yohimbine is a neural regulator of physiological color changes of the Medaka melanophores (Morishita, 1987). Yohimbine and Chlorpromazine caused morphological collar changes in swimming larvae, by continuous treatment from an early developmental stages in this experiment. It is difficult to explain these results because of a simple mechanism, but at

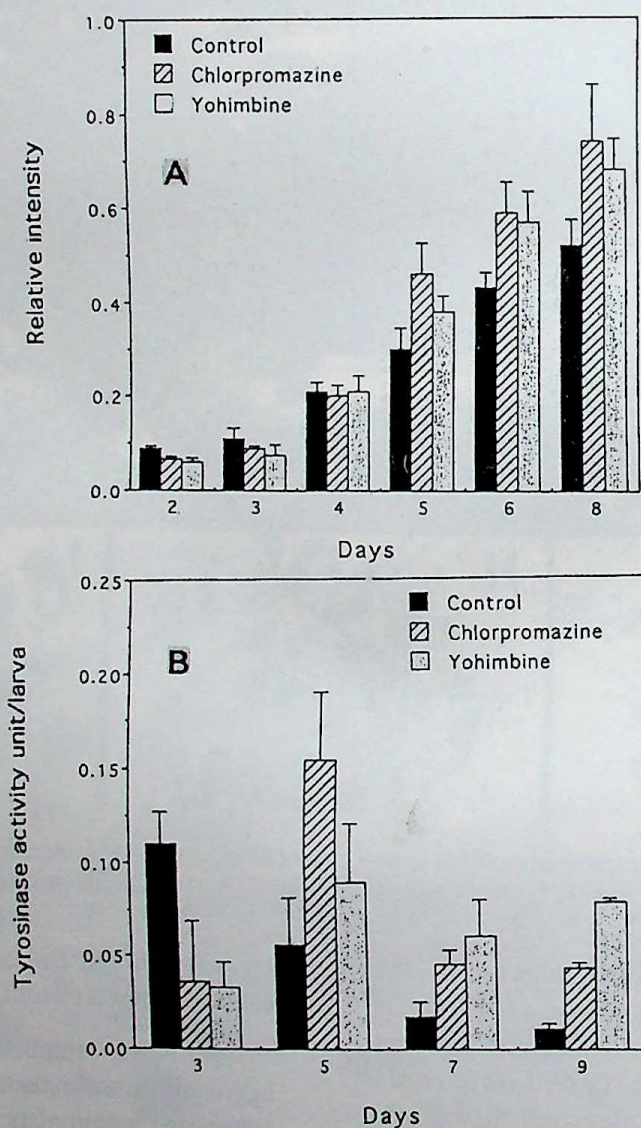


Fig. 3 : A : Relative intensity of melanin EPR signal of melanin to third peak of Mn^{++} ion. Values represent mean \pm SD $n=15-20$ $P < 0.05$ vs control. B : Tyrosinase activities of *Xenopus* larvae after hatch. Values represent mean \pm SD $n=20-24$ $P < 0.05$ vs control.

least skin darkened larvae appeared with neurochemical antagonists. A short period of the antagonists stimulation brings about physiological effects while prolonged exposure especially at

high concentration is conducive to morphological color change.

So far, we have only the above two antagonists' effects, which have produced

significant developmental abnormalities. However, there are some other agents that appear to have teratogenic effects, but it is difficult to measure what these effects are, and to date we have got to screen them. It is therefore necessary to further investigate the role of these exogenous chemical agents during amphibian development. Until now, little work has been done on this, but we feel that our methods in the future, will produce some useful new information on how environmental conditions may effect the early development of amphibians.

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Comparison of growth performance of *Lolium perenne* L., *Dactylis glomerata* L. and *Agropyron elongatum* (Host.) P. Beauv. for erosion control in Turkey¹.

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Abstract : This study was carried out in plastic containers to compare growth performances of perennial ryegrass, orchardgrass and tall wheatgrass to be given priority in revegetation studies in Turkey. Three pre-germinated seeds of each grass species were planted separately into the soil in the black plastic containers. Seedlings were harvested 2, 4 and 6 months after planting pre-germinated seeds and measured for percentage of seedling emergence, rooting depth, height growth, leaf and tiller development and shoot and root weights. Germination percentage was 97.8% for perennial ryegrass, 64.1% for orchardgrass and 11.6% for tall wheatgrass and perennial ryegrass had the greatest whereas tall wheatgrass had the lowest seedling emergence. Two months old rooting depth was 25.66 cm for perennial ryegrass, 20.56 cm for orchardgrass and 30.10 cm for tall wheatgrass. At the end of the study, perennial ryegrass developed about 104 tillers per plant while they were 21.4 and 36.6 tillers per plant for orchardgrass and tall wheatgrass, respectively. Orchardgrass produced greater shoot and root biomasses than tall wheatgrass and similar to perennial ryegrass. All these meant that perennial ryegrass had a better growth performance than orchardgrass and tall wheatgrass to be used for erosion control.

Key words : Tall wheatgrass, Orchardgrass, Erosion control, Perennial ryegrass, Rooting depth.

Introduction

Soil erosion is a serious problem and takes place in many parts of the world with different intensities depending on traditional land-use practices and its history. An erosion process begins with a sheet or surface erosion, which turns to rill, and then gully erosions in advance levels (Brady, 1990; Brooks *et al.*, 1996). Therefore, controlling sheet erosion is an easy and economical work to successfully prevent soil loss. Vegetative and mechanical techniques are used most commonly for sheet or surface erosion control (Brooks *et al.*, 1996). Mechanical techniques are very expensive and have a limited life expectancy depending on the amount of runoff and sediment production of the site (Morgan, 1990; Brooks *et al.*, 1996). Preference of mechanical or vegetative measures depends upon the long-term and short-term objectives. Mechanical treatments can achieve the goals in a short period compared to other methods but they also need follow-up maintenance. They are

generally preferred for short-term objectives and when site conditions limit plant growth. In contrast, vegetative measures take long time and are much more feasible to accomplish the goal. Moreover, establishment and maintenance of a vegetation cover can improve infiltration rate, reduce rainfall impact, increase surface roughness, porosity and water holding capacity of soil and thus reduce the velocity of surface runoff. Therefore, the most effective method to stabilize the soil surface is to establish a vegetation cover as quickly as possible on the site (Brooks *et al.*, 1996). The amount of root density in top soil and foliage or canopy cover on the soil surface are important factors to decrease soil loss by surface flow and also for protection of land surface from raindrop impact through interception of rainfall (Schwab *et al.*, 1993). Therefore, rapid establishment and growth with a deep, dense root system and high foliage canopy are the most effective and desirable plant characteristics in the land rehabilitation studies (Morgan, 1990). In other words, a plant, which is selected to be used

for soil conservation purposes, should have a dense and deep root system, dense foliage structure, early germination and growth habit and must be drought resistant due to low water holding capacity of soils in degraded sites.

In Turkey, since vast amount of soil erosion takes place in semi-arid regions of the country because of heavy grazing of the rangelands, drought resistant plant species must be concerned in erosion studies. Forest service gives priority to mechanical techniques like terraces and often uses a combination of terraces and plantation of woody species. In erosion studies, when vegetative treatments are applied, priority is given especially to the trees compared to grasses, forbs and shrub species in Turkey. Even after plantation of tree saplings, soil erosion takes place for a while until land surface is covered by tree canopies and adequate litter accumulates. In contrast, it takes much less time for grasses to germinate and grow to establish a plant cover on the land to protect soil surface from raindrop impact.

Various perennial grass species grow in Turkey but their use in soil erosion treatments is not common. Additionally, a limited knowledge is available about these grasses in terms of the land rehabilitation studies because individual species have different effectiveness for soil conservation (Morgan, 1990; Schwab *et al.*, 1993). Therefore, knowledge of available plants is very important and necessary to carry out successful erosion studies.

In many researches, different grass species have been studied to investigate the effect of defoliation on plant growth but no comparative study is available concerning the growth performance of Turkey's native, perennial, cool-season grasses, perennial ryegrass (*Lolium perenne* L.), orchardgrass (*Dactylis glomerata* L.) and tall wheatgrass (*Agropyron elongatum* (Host.) P. Beauv). Therefore, the objectives of this study were to determine how these species differed from each other for seed germination, seedling emergence, leaf and tiller development, height growth, rooting depth and shoot and root biomass

productions; and to find the most suitable grass species for stabilization of the land surface in erosion treatments.

Material and Methods

Plant species : Perennial ryegrass (*Lolium perenne* L.), orchardgrass (*Dactylis glomerata* L.) and tall wheatgrass (*Agropyron elongatum* (Host.) P. Beauv) were selected for this experiment based on their availability as revegetation species and good forage values for range animals (Uluocak, 1979). All these species are a cool-season, perennial bunchgrasses, drought resistant and well adapted to wide range of soil types and low to high soil moisture conditions (Uluocak, 1979). They grow everywhere in Turkey. Also, these grasses develop a fibrous, deep and dense root system and dense canopy that make them effective for reducing soil erosion (Uluocak, 1979; Wasser, 1982).

Seed germination, sowing pre-germinated seeds and harvesting : Seeds of perennial ryegrass, orchardgrass and tall wheatgrass were obtained from Erzurum Research Station which lies in Eastern Anatolian part of the country. Four replications of 100 seeds of each species were placed on moistened (with distilled water) filter paper in Petri dishes to estimate germination percentages. Petri dishes were placed in a germination chamber with a night/day temperature regime of 10°C / 20°C and a 12-h photoperiod. When coleptile had emerged and radicle had elongated to 5 mm seeds were considered germinated (Copeland, 1978), the germinated seeds were counted on daily basis over a month. In addition, in the same controlled environment chamber and at the same time enough seeds were placed in Petri dishes for each species to have pre-germinated seeds to be planted into the soil in plastic containers.

Large black plastic containers (38 cm diameter, 50 cm deep) with three drainage holes (1 cm diameter) at the bottom were filled with a sandy loam soil and put the field under outdoor conditions at Atatürk Arboretum which is a collaborative division of the Faculty of Forestry,

Istanbul University and the Turkish Forest Service. The soil was watered to field capacity based on soil moisture measurement in the laboratory before planting seedlings. When the primary root reached to 1 mm in length, three pre-germinated seeds of each species were taken from Petri dishes and planted separately in proper depths into the soil in the black plastic containers on mid March of 2001. To minimize interactions between developing seedlings, seeds were a minimum of 22 cm apart in the soil.

According to Thornthwaite method, the climate of the study site is humid, mesothermal oceanic with a moderate water deficit in summer. Average annual precipitation is about 1094 mm and mainly falls from October to April. During the experiment, average monthly temperatures were higher (Fig.1) and average monthly precipitations were lower (Fig.2) than long-term averages (DMIGM, 2001). Therefore, due to high temperatures and low rainfalls compared to long term averages and the holes at the bottom of the plastic containers all treatments received 3 mm supplemental water twice a week in the absence of weekly rainfall. Treatments were monitored for seedling emergence and survival and emerging seedlings were grouped into cohorts. Seedlings were harvested 2, 4 and 6 months after pre-germinated seeds were sowed into the soil. Containers were placed on their sides and soil was carefully removed from the containers over a screen. Soil was washed from the root systems of seedlings. Root length was measured using a ruler only on first harvest, 2 months after sowing pre-germinated seeds but wasn't measured on later harvests since roots of grass species had already reached the bottom of the containers. Additionally, seedlings were measured for height growth and the numbers of tillers and leaves were counted for each plant. After harvesting seedlings, their root and shoot, biomasses were dried in an oven at 65°C for 48 h and weighed on per plant basis. Due to entanglement of the roots of grasses in some containers, it was hard to separate roots of individual species growing in the same containers. Instead of measuring root biomass of individual species, an average root

biomass was calculated for each container, especially on last harvest, 6 months after sowing pre-germinated seeds. In these cases, total weight of root mass was weighed for each container and then divided by the numbers of grasses growing in the same container.

Experimental design and analysis : The experiment was arranged in a two-way factorial, randomized, split-plot design with eight replications for each treatment. The factors were plant species (perennial ryegrass, orchardgrass and tall wheatgrass) and harvests (2, 4 and 6 months after sowing pre-germinated seeds). Plant species were the whole plot and harvests were split plot. The data for seedling emergence, rooting depth, height growth, numbers of tillers and leaves, root and shoot biomasses were square root-transformed while the data for germination percentages of seeds were arcsine-transformed prior to using analysis of variance and means were compared using Turkey's test ($P < 0.05$) (Zar, 1996). During the experiment, only one perennial ryegrass seedling died. Therefore, seedling survival analysis was not conducted.

Results and Discussion

Seedling emergence, the numbers of tillers and leaves, rooting depth (only for first two months), height growth, shoot and root biomasses productions of perennial ryegrass, orchardgrass and tall wheatgrass in a field study and germination percentages of their seeds in a laboratory study were compared to determine the most favorite grass species to use in the revegetation treatments in Turkey.

Results showed that species differed significantly for seed germination and seedling emergence ($P < 0.05$). Perennial ryegrass had the greatest while tall wheatgrass had the lowest germination percentage and seedling emergence (Table 1). Even though seeds of neither species require pretreatment for germination, tall wheatgrass seeds had very low germination percentage. Though Wasser (1982) reported that tall wheatgrass seeds germinate in 21 days under ideal conditions, some experiments showed that

germination of tall wheatgrass seeds can be increased if the seeds are kept 16 hours in darkness at 20°C for 7 days (Senter *et al.*, 1975; Miller and Chapman, 1978). Low germination percentage of orchardgrass and tall wheatgrass may be attributed to low viability of seeds used in this study because seeds of neither species were tested for viability. Both grasses, which had poor germination percentages, had also very low

seedling emergence in the plastic containers under the field conditions as well. Although pre-germinated seeds were planted into the soil, tall wheatgrass had average one while perennial ryegrass had about three seedlings emerged in each container (Table 1). Perennial ryegrass seeds germinated earlier and faster than orchardgrass and tall wheatgrass seeds.

Table - 1 : Seed germination, seedling emergence and rooting depth of grass species.

Parameters	Grass species		
	Perennial rye grass	Orchardgrass	Tall wheatgrass
Germination percentage of seeds (%)	97.80 (3.47) ² a ³	64.10 (1.86) b	11.60 (2.49) c
Number of seedlings emerged per container	2.72 (0.01) a	1.11 (0.04) b	0.73 (0.04) b
Two months old rooting depth (cm)	25.66 (0.12) a	20.56 (0.49) a	30.10 (0.56) a

²Numbers in parentheses are standard errors of the means.

³Means with different letters are significantly different between grass species in each row at alpha 0.05 levels as determined by Tukey's test.

Majority of seeds started to germinate 3, 5 and 7 days after seeds were placed into Petri dishes and it lasted about 6, 10 and 15 days for perennial ryegrass, orchardgrass and tall wheatgrass, respectively. Seedling emergence of the species followed similar pattern to their germination percentages. Majority of perennial ryegrass seedlings emerged in 10 days and lasted about one month. Seedling emergences of orchardgrass and tall wheatgrass were slower than those of perennial ryegrass. Seedling emergence of orchardgrass lasted about 40 days while those of tall wheatgrass lasted 50 days after pre-germinated seeds were planted into the soil.

In contrast to germination percentages and seedling emergences, although tall wheatgrass developed the deepest root system (Table 1), grass species did not show significant differences for average overall 2 months old rooting depth. In order to detect differences among the species for rooting depth, if there were at earlier weeks, the grasses should have been harvested and examined earlier than 2 months after planting. In a defoliation study, Evans (1973) compared growth performance of six pasture species and found that perennial ryegrass

had a greater total root length than orchardgrass 26 days after seed germination as observed in this study 2 months after sowing pre-germinated seeds. A dense and deeper root system especially in the early stages of plant growth is a desirable plant characteristic for erosion control in arid and semi-arid regions. This is because rapid initial development of roots enables seedlings to acquire necessary resources for growth and reaches the deeper soil layers before hot-dry summer approaching in often-stressful disturbed environments (Coyne and Bradford, 1985; Newman and Moser, 1988).

In addition, having a greater root length provides some advantages to the plants. In a greenhouse study with sandy loam soil, Aguirre and Johnson (1991) found that rapid leaf and tiller development and greater leaf area were associated with greater total root length.

Average overall height growth of perennial ryegrass was low and significantly differed from both orchardgrass and tall wheatgrass, which had similar height growth (Table 2). All grass species had similar height growth two months after planting pre-germinated

seeds but orchardgrass on the second harvest and perennial ryegrass on the last harvest differed significantly from other two species ($P < 0.05$) (Table 2). Regardless of the species, height

growths were faster until second harvest but later slowed down toward end of the study for perennial ryegrass and orchardgrass. In contrast, height growth of tall wheatgrass

Table – 2 : Number of leaves and tillers, plant height, shoot and root weights of the grass species.

	Perennial rye grass	Orchardgrass	Tall wheatgrass
Plant height (cm)			
1st harvest	7.79 (0.06) ⁴ a ⁵	8.70 (0.23) a	8.12 (0.17) a
2nd harvest	14.86 (0.13) b	28.08 (0.35) c	19.13 (0.40) b
3rd harvest	18.56 (0.07) b	33.04 (0.40) c	37.11 (0.36) c
Number of leaves			
1st harvest	29.62 (0.18) a	13.84 (0.53) a	8.68 (0.28) a
2nd harvest	465.38 (1.31) b	124.18 (1.78) c	69.97 (0.98) c
3rd harvest	807.78 (1.57) d	238.20 (1.36) ce	227.23 (2.11) e
Number of tillers			
1st harvest	7.85 (0.10) a	3.61 (0.26) a	2.81 (0.22) a
2nd harvest	97.32 (0.51) b	22.84 (0.72) cd	16.53 (0.48) a
3rd harvest	103.86 (0.48) b	21.40 (0.37) c	36.60 (0.80) c
Shoot weight (g)			
1st harvest	0.11 (0.02) a	0.08 (0.06) a	0.04 (0.02) a
2nd harvest	4.31 (0.18) b	7.12 (0.46) b	1.08 (0.21) a
3rd harvest	7.38 (0.16) b	10.61 (0.40) b	2.29 (0.57) b
Root weight (g)			
1st harvest	0.10 (0.02) a	0.05 (0.04) a	0.03 (0.02) a
2nd harvest	7.55 (0.21) b	6.14 (0.47) b	0.41 (0.12) a
3rd harvest	7.19 (0.19) b	8.84 (0.51) b	8.32 (0.40) b

⁴ Numbers in parentheses are standard errors of the means.

⁵ Means with different letters are significantly different in each row between species and each column for the same species at alpha 0.05 level for each plant characteristics as determined by Tukey's test.

increased steadily along the research (Table 2). At the end of the study period, tall wheatgrass achieved greatest height growth while perennial ryegrass had lowest growth. However, tall wheatgrass and orchardgrass weren't significantly different for height growth. Neither species in this study showed good performance for height growth as reported in the literature (Uluocak, 1979; Wasser, 1982). The size of the containers would have an effect on plants growth performance (Bainbridge *et al.*, 1995). The volume of the containers most probably weren't big and deep enough for six months research

period because any inhibition of root growth can cause a decrease in the growth of above ground parts of plants such as tillers and leaves, plant height and shoot biomass (Jacques and Edmond, 1952). But some experiments showed that the containers with 50 cm depth would be acceptable for these grass species because these species have high branched, shallow root system and make most of their root development in the upper part of the soil but can extend to below the soil surface (USDA Forest Service, 2001; Hannaway *et al.*, 1999). Additionally, Weaver (1950) classified a pasture into high, medium, fair and low grades

depending on existence of amount of climax grasses and found that about 85% of the total root weight occurred in 26 cm soil depth in the pasture under different grazing intensities. Besides size of the container, its color could also affect plant growth. Since black-colored containers absorb

solar energy and can increase the temperature of growing medium that may cause water loss through evapotranspiration. In a similar study, Brown (1982) showed that temperature of growing medium in dark-colored containers was 7°C greater than that in white-colored containers.

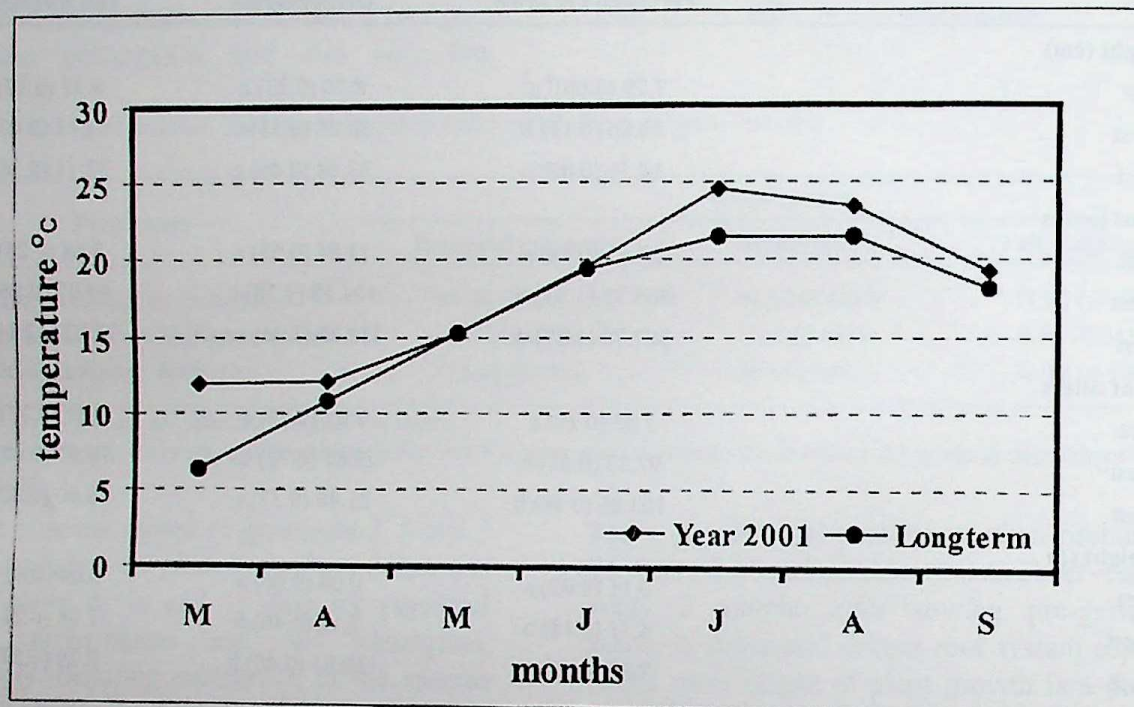


Fig. 1 : Average monthly temperatures from March to September.

An average overall leaf production of the species was significantly different but those of orchardgrass and tall wheatgrass were similar as seen in Table 2. Species differed significantly for leaf production on each harvest except for first harvest ($P < 0.05$). In contrast to height growth, perennial ryegrass had the greatest leaf development without exception on each harvest while tall wheatgrass had the lowest, which increased rapidly throughout the investigation (Table 2). Leaf production performance of perennial ryegrass was similar to those found by Haggar (1979) in another experiment. He observed that perennial ryegrass developed about 25 leaves per plant 10 weeks after sowing the seeds. The same grass species produced about 30 leaves 2 months after planting pre-germinated seeds in this study (Table 2).

Species performances for tiller development were also significantly different ($P < 0.05$). In a given period, perennial ryegrass developed more tillers than other two grasses and had 8 tillers per plant 2 months after planting pre-germinated seeds (Table 2). The result for perennial ryegrass was close to 6 tillers per plant reported in another study by Haggar (1979) 10 weeks after sowing the seeds. Perennial ryegrass developed a denser ground cover that grew closer to the soil surface as pointed out by Hannaway *et al* (1999) than orchardgrass and tall wheatgrass to intercept raindrops before they reach to the soil surface. On the other hand, although average overall shoot biomass productions of the species were significantly different (Table 2), no significant differences were observed between perennial ryegrass and other two grasses. A significant difference occurred only between tall

wheatgrass and orchardgrass. Tall wheatgrass had the lowest shoot weight and its trend of shoot biomass production was slow through the study while orchardgrass had greatest biomass weight. The interaction between grass species and harvest dates was also significant for production of shoot

biomass. Some differences occurred between the grasses for shoot weight only on the second harvest. Even though orchardgrass produced much more shoot biomass on each harvest except for first harvest compared to the other grasses, it

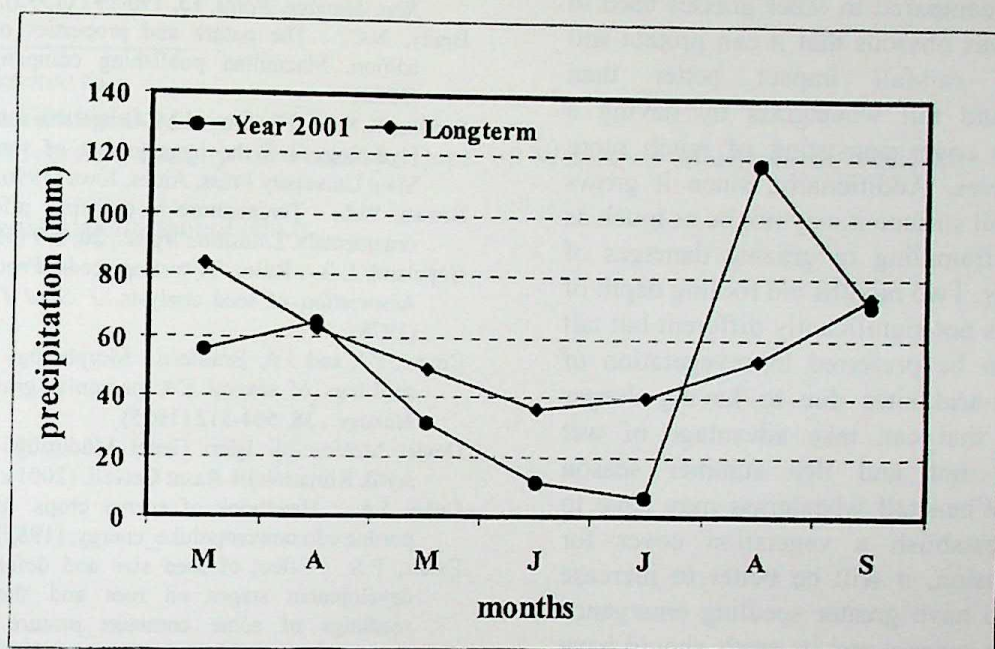


Fig. 2 : Average monthly precipitations from March to September.

was not significantly different from perennial ryegrass for all harvests (Table 2). In contrast, in a comparative study, Evans (1973) studied six pasture species under New Zealand pasture conditions and found that perennial ryegrass produced greater shoot weight than orchardgrass 26 days after seed germination.

Results revealed that even though differences exist between average overall root biomass weights of the species, they were not significant but the interactions between grass species and harvests were statistically significant. The trend of root biomass productions of the species was exactly similar to their shoot biomass productions. As happened in shoot biomass productions, orchardgrass and perennial ryegrass developed similar amount of root biomass and were greater than those of tall wheatgrass only on second harvest in contrast to the results of the

experiment carried out by Evans (1973). Root biomass production of the grasses were similar 2 and 6 months after seedlings were planted but they showed only significant differences 4 months after planting (Table 2). Root biomass increased within 4 months for perennial ryegrass and orchardgrass and 4 months after planting pre-germinated seeds for tall wheatgrass. That's, root growth of tall wheatgrass was slower than other two grasses as seen in shoot biomass production and tiller development. In addition to greater shoot weight, results showed that although there was no significant difference between species for overall average root weights; orchardgrass had greater root weight compared to perennial ryegrass and tall wheatgrass as well.

Results showed that the order of priority from high to low grade that may be given to the species in revegetation studies should be

perennial ryegrass, orchardgrass and tall wheatgrass because perennial ryegrass had a better growth performance than orchardgrass and tall wheatgrass. Due to higher seed germination percentage and seedling emergence, stands of perennial ryegrass can establish quickly in a shorter period compared to other grasses used in this study. It was obvious that it can protect soil surface from rainfall impact better than orchardgrass and tall wheatgrass by having a denser canopy cover consisting of much more tillers and leaves. Additionally, since it grows closer to the soil surface it may not be as much as vulnerable to trampling or grazing damages of grazing animals. Two months old rooting depth of the species was not significantly different but tall wheatgrass can be preferred in revegetation of arid and semi-arid sites due to having deeper rooting depth that can take advantage of wet spring before hot and dry summer season approaching. When tall wheatgrass may have to be used to establish a vegetation cover for controlling erosion, it will be better to increase seeding rate to have greater seedling emergence and chance for success and its seeds should have pretreatment to increase germination. The effect of competition on growth performance of plant species was not examined in this study. Therefore, it is unknown how growth performance of these grasses will be affected due to competition when they grow together with other grass and/or forb species in dense stands. This study includes one vegetation period and it was conducted in containers. In addition, to observe regeneration capacities of the species additional experiments are needed to be conducted in field conditions for a longer period.

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Complex dynamics of toxin producing algal species and primary productivity in two water ponds of Faizabad.

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Abstract : In order to develop a method of predicting and assessing pond eutrophication, which is a serious environmental problem, and to propose effective measure of improvement of water quality. The present study was performed to measure water quality variables, primary productivity, chl-a and biomass of toxin producing algal species and fish production. High nutrient influx and toxin producing algal species have been observed during June 1999 to May 2000 in two water bodies [Girija Kund (A) and Maqubara pond (B)] of Faizabad. The maximum chl-a concentration, toxins producing algal species biomass were found to be 415.00 and 515.00 in pond A, while 451.00 $\mu\text{g/l}$ and 541.22 mg/l in pond B, respectively in the case of *Microcystis aeruginosa*. Ecological parameters to evaluate GPP, NPP and CR were found to be 297.00, 134.000 and 182.00 mgCm^3/h in summer season in pond B, respectively which is higher than pond A. A poor association existed between chl-a and GPP. Temporal variation (Photosynthetic rate) in A_{max} & P_{max} was also observed to evaluate the productivity of pond. Annual fish production potential of the ponds A and B has been estimated to be around 342.00 Kg and 204.00 Kg, respectively which may be due to the presence of toxin producing algal species.

Key words : Toxin producing algal species, Biomass, Chlorophyll-a, Primary productivity fish production.

Introduction

Natural populations are linked by multifold interactions with one another and with their environment. This yields a dynamic pattern of decline and recovery of the populations. This becomes more complex when inputs of toxic substances especially toxin producing algal species occur. Usually, substances of concern affecting ecosystem are repeatedly discharged and / or persistent. As ecological systems are hierarchically structured, a toxin stress may affect all level of biological organization, including the community (Landis *et al.*, 1996; Matthews *et al.*, 1996).

Cyanobacterial poisoning in aquatic environment is of considerable interest, which dominate the heavy phytoplankton in many fresh and saline water. Although in a healthy balanced aquatic ecosystem, toxin producing algal species are small but important component of the natural plankton population, which can turn algal blooms a green and potentially poisonous.

The importance of algal dynamics, particularly their response to environmental changes and nutrient fluctuations has been suggested by several investigators (Duncan *et al.* 2000; Dwivedi and Pandey, 2001; 2002 a; 2002 b; Pandey and Dwivedi, 2002).

Measurement of photosynthetic or primary productivity is important in food chain studies. The daily and seasonal carbon flow of a system forms the basis for the structure of the annual pyramid and can be used to assess the trophic status and fish production potential of aquatic ecosystem (Ahmad and Singh, 1987; Lund *et al.*, 2000; Prakasam and Joseph, 2000). Though information on primary production from Indian fresh water is abundant (Sreenivasan, 1963; Tallberg *et al.*, 1999; Krishna Rao and Shakuntala, 1999), concurrent data on toxin producing algal species biomass and chl-a is scarce.

In the present study primary productivity of toxin producing algal species and their inter-

relationship with biomass and chl-a were explored in order to evaluate the possibilities to improve the water quality for substantial pisciculture in two ponds, Girija Kund (A) and Maqubara Pond (B) of Faizabad, India.

Materials and Methods

Physico-chemical and biological characteristics were studied seasonally [summer (February-May), Rainy (June-September) and winter (October- January) during the year, May 1999 to June 2000 in two ponds [Girija Kund (A) and Maqubara Pond (B)] of Faizabad (Dwivedi and Pandey, 2001). Collection, preservation and counting of toxin producing algal species have been reported in earlier studies (Dwivedi and Pandey, 2001; 2002 a; and 2002 b).

The rate of primary production at the surface was estimated using in-situ light and dark bottle technique (Gaarder and Gran, 1927; Michael, 1986; APHA *et al.* 1999). Productivity values were obtained at every 4 h intervals from dawn to dusk. The rate of Gross Primary Productivity (GPP), Net Primary Productivity (NPP) and Community respiration rate (CR) were calculated according to the formula given by CIFRI (1969). Chlorophyll (chl-a) of the toxin producing algal species sample of the water ponds was determined by filtration through Whatman GF/C filter. The pigment was extracted in alkaline acetone. The filters were stored over a desiccant and deep frozen unit analysis, which was undertaken within 24 h (Krishna Rao and Shakuntala, 1999). The cell pellet was extracted twice in 80% acetone and then resuspended in a 0.2M sodium acetate buffer (pH 5.5). The absorbance was measured at 625, 678, 725 nm. Toxin producing algal species biomass was calculated from cell counts multiplied with average cell volumes estimated by assuming simple geometrical bodies and measuring the necessary dimension from about 40 cells / taxon. Assuming a biomass / volume relationship approximately 1 : 1, 1 mm³ biovolumes corresponds to a fresh weight of roughly 1 mg, or to 0.2 mg of dry weight. Correlation- coefficient

was done between toxin producing algal species and their Chl-a and biomass for the reality and significance of the results.

Results and Discussion

The salient features of chl-a, biomass and correlation coefficient (r) between toxin producing algal species and chl-a and biomass have been presented in Table 1, 2 and 3. Primary productivity in terms of GPP, NPP, CR and Temporal variation (Photosynthetic rate) in A_{max} & P_{max} were given in Table 4 and Figure 1, respectively.

In an earlier study, both ponds (A and B) were analysed for their physico-chemical parameters and it was found that these ponds exceeded the permissible limits of BIS and showed high eutrophication. In both ponds twelve toxin producing cyanobacterial algal communities (*Microcystis sp.*, *M. protocystis*, *M. aeruginosa*, *M. lotralis*, *M. incerta* *Oscillatoria sp.*, *O. princeps*, *O. limosa*, *Lyngbya sp.*, *L. majuscula*, *Nostoc sp.* and *Anabaena sp.*) were identified (Dwivedi and Pandey, 2001). The number of toxin producing algal species increased dramatically as 714 to 7900 and 614 to 8346 cells/ml in winter to summer season and rainy to summer season in pond A and B, respectively (Dwivedi and Pandey, 2001). In summer season the dominated species viz. *Microcystis sp.*, *M. protocystis*, *M. aeruginosa* were found in both ponds. In winter season, the toxin producing algal species were still high, but the dominant species were bacillariophyceae in pond B only (Dwivedi and Pandey, 2002 b).

The Chl-a concentration was relatively low as 90 and 87 µg/l during winter and rainy season thereafter the concentration rapidly increased as 415 and 451 µg/l, in summer season in Pond A and B, respectively (Table-1). It also reflects the maximum number of toxin producing algal species as 7900 and 8346 cells/ml in Pond A and B, respectively (Dwivedi and Pandey, 2001). This difference was due to a combination of factors such as nutrient concentration by evaporation and better nutrient replenishment.

Similar observation has been reported by Krishna Rao and Shakuntala (1999) from other reservoir.

The biomasses of 12 toxin-producing algal species were found to range from 106- 515

and 46.24 -541 mg/l in A and B pond, respectively for three seasons. Maximum toxins producing algal

Table - 1 : Chlorophyll-a of different toxin producing algal species in Girija Kund (A) and Maqubara pond (B).

Toxin producing algal sp.	Summer season (Feb – May)		Rainy season (June – Sep)		Winter season (Oct – Jan)	
	A	B	A	B	A	B
<i>Microcystis sp.</i>	405.70	446.07	265.00	296.01	245.78	385.00
<i>M. protocystis</i>	365.00	431.01	195.00	185.00	165.00	125.00
<i>M. aeruginosa</i>	415.00	451.00	215.00	125.02	151.02	251.67
<i>M. lotralis</i>	320.01	370.00	170.00	87.00	132.00	152.00
<i>M. incerta</i>	290.00	298.02	314.00	140.11	90.01	100.00
<i>Oscillatoria sp.</i>	199.69	269.00	109.46	146.00	119.00	126.00
<i>O. princeps</i>	229.29	292.00	109.00	151.00	112.00	129.90
<i>O. limosa</i>	318.00	381.00	283.03	278.00	183.07	301.02
<i>Lyngbya sp.</i>	214.00	314.01	142.00	-	114.00	104.00
<i>L. majuscula</i>	241.00	412.00	-	-	124.00	134.01
<i>Nostoc</i>	242.04	282.00	141.00	271.60	242.00	261.24
<i>Anabaena</i>	402.01	448.00	114.80	148.00	219.00	224.84

Values (Mean) of Chlorophyll-a expressed as µg/l of three replicates. (-), represents as absence of values because species were not present.

Table - 2 : Biomass of toxin producing algal species in Girija Kund (A) and Maqubara pond (B).

Toxin producing algal sp.	Summer season (Feb – May)		Rainy season (June – Sep)		Winter season (Oct – Jan)	
	A	B	A	B	A	B
<i>Microcystis sp.</i>	493.99	493.91	231.91	274.19	231.10	345.00
<i>M. protocystis</i>	308.00	378.01	128.00	120.00	112.00	142.00
<i>M. aeruginosa</i>	515.00	541.00	248.00	128.02	134.02	398.67
<i>M. lotralis</i>	398.01	380.00	108.12	64.00	178.00	181.00
<i>M. incerta</i>	319.00	382.02	438.00	132.11	149.01	156.00
<i>Oscillatoria sp.</i>	299.69	321.00	119.06	91.00	106.00	166.00
<i>O. priceps</i>	318.29	381.00	121.00	122.00	146.00	204.46
<i>O. limosa</i>	411.00	441.00	336.03	301.00	301.07	96.02
<i>Lyngbya sp.</i>	326.10	368.01	-	-	146.00	99.00
<i>L. majuscula</i>	398.00	399.00	218.12	-	318.00	91.18
<i>Nostoc</i>	322.21	312.00	163.00	165.60	334.00	46.24
<i>Anabaena</i>	484.21	492.00	182.00	198.00	242.00	68.32

Values (Mean) of biomass expressed as mg/l of three replicates. (-), represents as absence of values because species were not present.

species biomass was found to be in summer season in both ponds (Table 2). Tallberg *et al.* (1999) have also noticed the same pattern in eutrophicated lake.

Amongst 12 toxin producing algal species, six species (*Microcystis sp.*, *M. protocystis*, *M. aeruginosa*, *M. lotralis*, *Oscillatoria limosa*, and *Anabaena sp.*) were found to be dominant species as its numerical

abundance (cell/ml) was relatively high throughout the study period in pond B than pond A. The biomass peak of *Microcystis sp.*, *M. protocystis*, *M. aeruginosa*, *Oscillatoria limosa* were also common in both ponds. In pond, A *Microcystis species* constitute 50% of the biomass from Feb-May and in pond B toxin producing algal species share of the biomass rose towards the end of summer season (>50%) in Oct-May.

Most of toxin-producing algal species found in both ponds belong to the genera *Microcystis*, *Anabaena*, *Oscillatoria*, *Lyngbya* and *Nostoc* and dominated in pond B.

Table-3 indicates certain biological indicator (chl-a and biomass) of toxin producing

algal species and their complex dynamics statistical relationship (Correlation-coefficient). The highest enumeration of toxin producing algal species was highly correlated with the Chl-a ($r=0.96$; $P<0.01$) and Biomass ($r=0.88$, $P<0.01$) in

Table – 3 : Correlation -coefficient (r) values between toxin producing algal species and chl-a and biomass recorded from the Girija Kund (A) and Maqubara pond (B).

Species	Chlorophyll-a		Biomass	
	A	B	A	B
1. <i>Microcystis</i>	0.93	0.96	0.82	0.88
2. <i>M. protocystis</i>	0.67	0.67	0.41	0.53
3 <i>M. aeruginosa</i>	0.45	0.78	0.41	0.78
4. <i>M. lotralis</i>	0.12	0.42	0.51	0.87
5. <i>M. incerta</i>	0.31	0.39	0.28	0.58
6. <i>Oscillatoria sp.</i>	0.21	0.31	0.31	0.42
7. <i>O. princeps</i>	0.69	0.21	0.38	0.39
8. <i>O. limosa</i>	0.31	0.51	0.22	0.20
9. <i>Lyngbya sp.</i>	0.21	0.19	0.22	0.20
10. <i>L. majuscula</i>	0.16	0.21	0.41	0.23
11. <i>Nostoc sp.</i>	0.21	0.39	0.41	0.61
12. <i>Anabaena sp.</i>	0.42	0.62	0.66	0.51

Table – 4 : The gross primary productivity (GPP), net primary productivity (NPP), community respiration (CR) rate, ratio of net primary productivity (NP : GP), ratio of productivity and respiration rate (P : R) and percentage of respiration in gross production in Girija Kund (A) and Maqubara Pond (B)

Parameters	Summer season		Rainy season		Winter season	
	A	B	A	B	A	B
Gross primary productivity (mg cm ³ /h)	258.00	297.00	225.00	205.00	174.45	256.00
Net primary productivity (mg cm ³ /h)	111.25	134.00	94.00	102.00	125.00	110.00
Community respiration rate (mg cm ³ /h)	156.00	182.00	164.00	147.00	122.00	89.42
N.P. : G.P. ratio	0.44	0.46	0.41	0.50	0.60	0.43
P : R	0.71	0.76	0.64	0.81	1.18	0.67
Gross production % respiration	0.60	0.61	0.65	0.59	0.51	0.64

both ponds (A and B) in summer season. Similar observation has been reported by Fromme *et al.* (2000) from Berlin water bodies.

The correlation coefficient between toxin producing algal species (*Microcystis sp.*, *M. protocystis*, *M. aeruginosa*, *O. princeps*, and *Microcystis sp.*, *M. protocystis*, *M. aeruginosa* & *Anabaena sp.* and *Oscillatoria limosa*, *Lyngbya sp.*, *L. majuscula*, *Nostoc sp.* and *Anabaena sp.*) with chl-a was highly positive in both ponds. In

the present study except *Microcystis sp.* *M. protocystis*, *Oscillatoria*, *O. princeps*, *Lyngbya sp.*, all toxin producing algal species showed highly positive correlation with biomass in both ponds. Lund, *et al.* (2000) and Oh *et al.* (2001) reported that the transparency was negatively correlated with Chl-a concentration, toxin producing algal species biomass from other reservoirs.

Dynamics of toxin producing algal species.

Although pond A is small but without any heterogeneity such as entry point of sewage and other sources, while in pond B spatial variations exist. In the present study, productivity measurement was found to fluctuate throughout

the study period in both ponds (Table-4). The GPP depicted a declining trend from summer to winter season as 258.00 to 17.45 and 297 to 256 mgCm^3/h in pond A and B, respectively. The trend of seasonal variations of NPP value was not

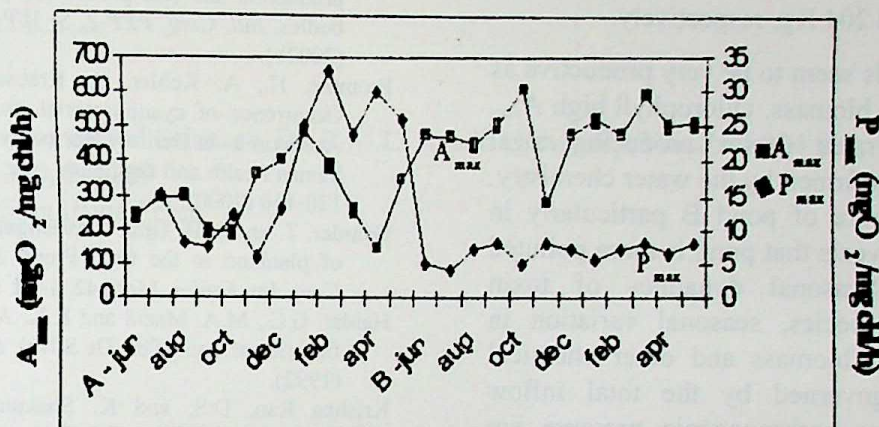


Fig. 1 : Temporal variation in A_{\max} and P_{\max} in Girija Kund (A) and Maqubara Kund (B).

similar to that of GPP showing the highest value during winter (125.00) and summer (134.00 $\text{mg Cm}^3/\text{h}$) season in pond A and B, respectively and least in summer as 94.00 and 102.00 $\text{mg Cm}^3/\text{h}$ in both ponds. CR value ranged from 89.42 to 156.00 $\text{mg Cm}^3/\text{h}$ in pond A, while pond B exhibited greater value (122.00 to 182.00 $\text{mg Cm}^3/\text{h}$). The ratio of NPP to GPP never exceeded 0.60 in all seasons in both ponds. The percentage of respiration rate to GPP ranged from 0.51-0.65 in both ponds. It always remained above 50%, the level that has been suggested for polluted water bodies (Ramarao *et al.*, 1979). Light saturated rate of photosynthesis unit volume (A_{\max}) may be regarded as a measure of the capacity of the pond to produce and sustain algae (Westlake, 1980). Fig.-1 reveals that variation in A_{\max} and P_{\max} were found in both ponds. The A_{\max} value was found to be 490 and 610 $\text{mgC}/\text{m}^3/\text{h}$ in pond A and B, respectively due to higher algal biomass. Most of the production was confirmed as low variation between both ponds with a maximum in pond B in summer season. In both ponds lower production were characterized as per unit volume and absence of marked maxima (when the pond was stratified and had high water level). Thereafter, a significant change in the shape

occurred with a truncation of the period (summer season) marked maxima and higher production per unit volume coinciding with low water level and higher toxin producing algal biomass. These results are comparable with the findings of Westlake (1980) reported from the other lake.

Light saturated gross photosynthesis per unit chlorophyll (P_{\max}) varied widely from a low of 6 and 4, to a high of 33 and 8 $\text{O}_2/\text{mg chl-a}/\text{h}$ in A and B pond, respectively (Fig.-1). However, Westlake (1980) reported that the upper extreme was ranged from 18 to 37.7 mg in eutrophic IBP lakes. The sharp decline in P_{\max} from April to July in B pond, indicates resource limitation for maximum photosynthetic efficiency. Although during study period production was higher in pond B than pond A in summer season with fluctuations depending on seasons. Reports are available that in several reservoir which were characterized by wide water level fluctuations and rapid filling, an inverse relation between production and water level indicated high production in summer and low in rainy season (Nasar and Dutta Munshi, 1975; Haldar, 1992). Both ponds have not been brought under scientific management and the fisheries is dominated by the *Labeo rohita*, *Heteropneustes*

fossilis, *Clarias batrachus*, *Cirrhinus mrigala*, *Wallago attu*, *Glaser*, *Catla-catla* and *Macrobrachium malcomsonium* which contributed > 80% to the commercial fishery (Personal observation). The annual fish production of pond A and B has been estimated to be around 342 and 204 Kg, respectively.

Both ponds seem to be very productive as is evident by high biomass, chlorophyll high A_{max} and production rates (toxin producing algal species) and as confirmed by the water chemistry. High respiration rate of pond B particularly in summer season reveals that pond is more polluted than pond A. Seasonal dynamics of toxin producing algal species, seasonal variation in productivity, algal biomass and other chemical parameters are governed by the total inflow received, and thus anthropogenic pressure are important. These findings emphasizes the importance of catchment management to reduce nutrient inputs, in addition to in - pond control of nutrients (possibly through sediments reduction) as a part of a suite of approaches, including biomanipulation, for restoration of eutrophic reservoirs in general and ponds of Faizabad are particular.

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Dynamics of toxin producing algal species.

61

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Effects of rogar and endosulfan on the metabolism of fresh water sponge (*Spongilla lacustris*).

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Abstract : *Spongilla lacustris* were exposed to sub lethal concentrations of pesticides, rogar and endosulfan for one month period. Metabolites like carbohydrates, protein and enzymes like those that peroxidase and carbonic anhydrase were estimated in the experimental and control animals.

The results show, depletion of carbohydrates while protein elevated as the days progressed. Similarly an enzyme activity found to be decreased in exposed *Spongilla lacustris*.

Key words : *Spongilla lacustris*, Pesticides, Bio-monitoring.

Introduction

Numerous biochemical indices of stress have been proposed to assess the health of non-target organisms exposed to toxic chemicals in aquatic ecosystem (Nimmi, 1990). However it has been shown by many workers that apart from nervous tissue, blood, liver and gills may also contribute a good deal of information in the detection of toxic symptoms caused by certain groups of pesticides, Christensen and Tucker (1976) proposed quantitative changes in carbonic anhydrase activity in channel catfish, *Ictalurus punctatus* as a diagnostic sign of organophosphorus poisoning. Gabbott (1976) stated that the energy metabolisms like carbohydrates, lipids and protein; an organism for energy production uses fuels.

In India, researchers have started investigating the effects of various pesticides on biochemical composition in invertebrates (Swami *et al.*, 1983; Mane *et al.*, 1986). Various authors using micro flora and fauna discuss the biomonitoring field (Rana, 1995). Tonapi (1964) stated that Indian freshwater sponges have suffered from neglect as far as its application in bio monitoring is concerned.

The present studies were designed to understand the impact of sub lethal concentrations

of rogar and endosulfan on protein, carbohydrate and enzymes viz. peroxidase and carbonic anhydrase in freshwater *S. lacustris* with an exposure period of 30 days.

Materials and Methods

Spongilla lacustris were collected from Latipada dam, Pimpalner, Dist-Dhule (M. S.). The dam is on Panzara River initiated from the small village Navapada near Mangi-Tungi hills of Western Ghats. Dam is located at the latitude 20°, 55" N and longitude at 74°-5'-30" E at 532 MSL.

The freshwater *S. lacustris* were acclimated to the laboratory conditions and exposed to sublethal concentration of rogar (Jaishree Agro. India. Ltd) and endosulfan (Excel India. Ltd). The LC₅₀ values were estimated by the graphical method (Litchfield and Wilcoxon, 1949). Control group of animals was maintained simultaneously.

The homogenate of the body of *S. lacustris* was used for estimation of protein (Waheed and Gupta, 1996) and carbohydrate (Dubious *et al.*, 1956), while enzymes peroxidase (Kashiwa and Atkinson, 1990) and carbonic anhydrase by Tripathi and Upadhyay (1998). The test was carried at the intervals of 2, 9, 16, 23 and 30 days.

Results and Discussion

The results on proximate composition of *S. lacustris* exposed to sub-lethal concentrations show variation in protein, carbohydrate, peroxidase and carbonic anhydrase, compared to control animal.

The protein content increased (Table 1 and 2) as the concentration increased and days progressed but the carbohydrates were depleted (Table 3 and 4). While the enzyme activities show inhibition in the exposed animal (Fig. 1 to 4).

The sponges content protein and amino acids but not free amino acid (Inskip and Cassidy, 1955). In sponge, the cell suspension for

aggregation was incubated in the medium containing ³H-isoleucine (1 μ ci/ml), the protein content was increased (Kantha and Mukherjee, 1978). The sponges content glucose and fructose (Gross and Rlugvie, 1968), in present study carbohydrates were depleted. In *L. marginalis*, exposed to malathion, decreased in the glycogen content reported by (Kabear Ahmad *et al.*, 1978). The change in carbohydrate metabolism of *L. marginalis* exposed to phosphamidon observed by (Sreenivasa *et al.*, 1983). The effect of insecticides on experimental animal caused changes in enzyme activities at higher concentrations. The enzyme activity inhibit to fish, exposed to the industrial effluents

Table-1 : Protein content (mg/100mg) of *Spongilla lacustris* exposed to rogar for 30 days.

	2 days	9 days	16 days	23 days	30 days	Average	S.D.
Control	0.13	0.14	0.21	0.18	0.2	0.172	± 0.035
1 ppm	0.15	0.13	0.15	0.14	0.17	0.148	± 0.014
2 ppm	0.13	0.19	0.29	0.17	0.22	0.2	± 0.06
3 ppm	0.22	0.21	0.14	0.15	0.18	0.18	± 0.035
4 ppm	0.27	0.18	0.16	0.15	0.15	0.182	± 0.050
5 ppm	0.17	0.17	0.22	0.18	0.18	0.184	± 0.020

Table-2 : Protein content (mg/100mg) of *Spongilla lacustris* exposed to endosulfan for 30 days.

	2 days	9 days	16 days	23 days	30 days	Average	S.D.
Control	0.18	0.13	0.27	0.21	0.22	0.202	± 0.051
1 ppm	0.06	0.06	0.25	0.16	0.18	0.142	± 0.081
2 ppm	0.04	0.02	0.15	0.13	0.15	0.098	± 0.063
3 ppm	0.02	0.04	0.13	0.25	0.26	0.14	± 0.112
4 ppm	0.005	0.05	0.14	0.16	0.18	0.107	± 0.075
5 ppm	0.015	0.06	0.16	0.28	0.28	0.159	± 0.122

Table-3 : Carbohydrates content (mg/100 mg) of *Spongilla lacustris* exposed to rogar for 30 days.

	2 days	9 days	16 days	23 days	30 days	Average	S.D.
Control	0.18	0.17	0.25	0.21	0.2	0.202	± 0.031
1 ppm	0.17	0.19	0.17	0.14	0.1	0.154	± 0.035
2 ppm	0.09	0.23	0.9	0.37	0.21	0.36	± 0.317
3 ppm	0.22	0.26	0.16	0.11	0.05	0.16	± 0.083
4 ppm	0.27	0.21	0.15	0.17	0.12	0.184	± 0.058
5 ppm	0.16	0.17	0.33	0.24	0.1	0.2	± 0.088

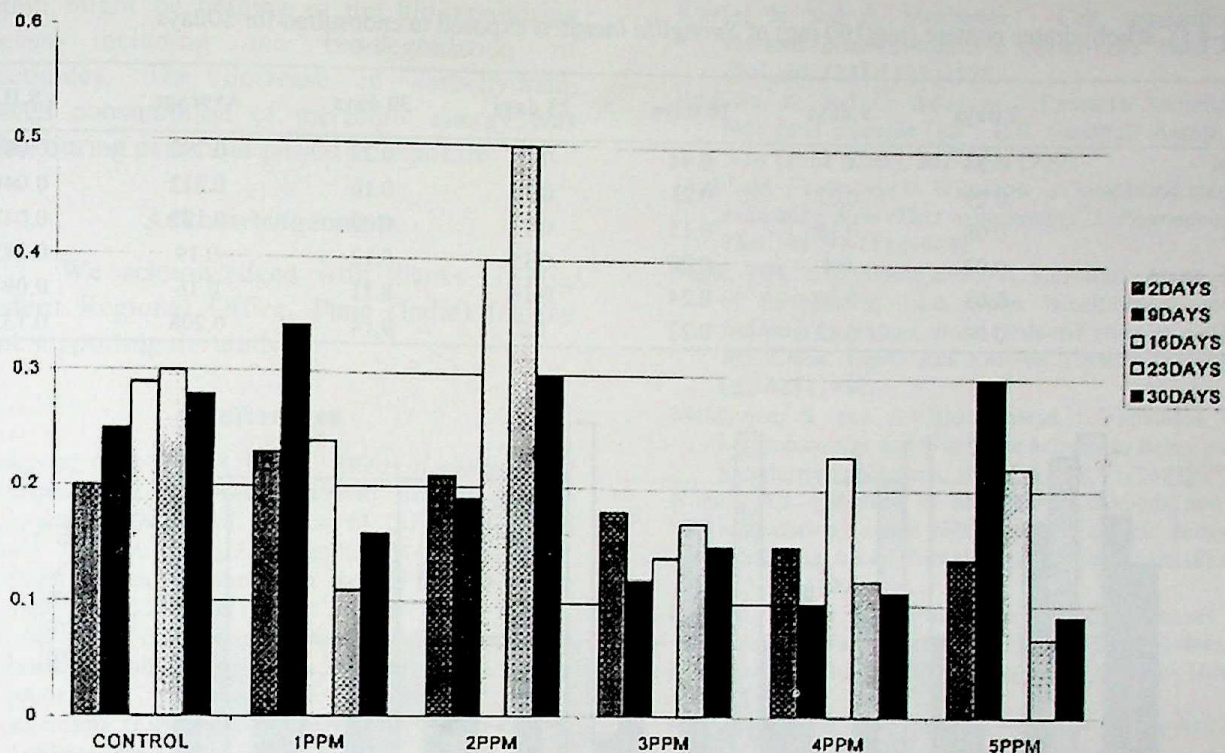
Effects of pesticides on metabolism of fresh water sponge.

Fig. 1 : Peroxide activity (μ moles/mg/min) of *Spongilla lacustris* exposed to rogar for 30 days.

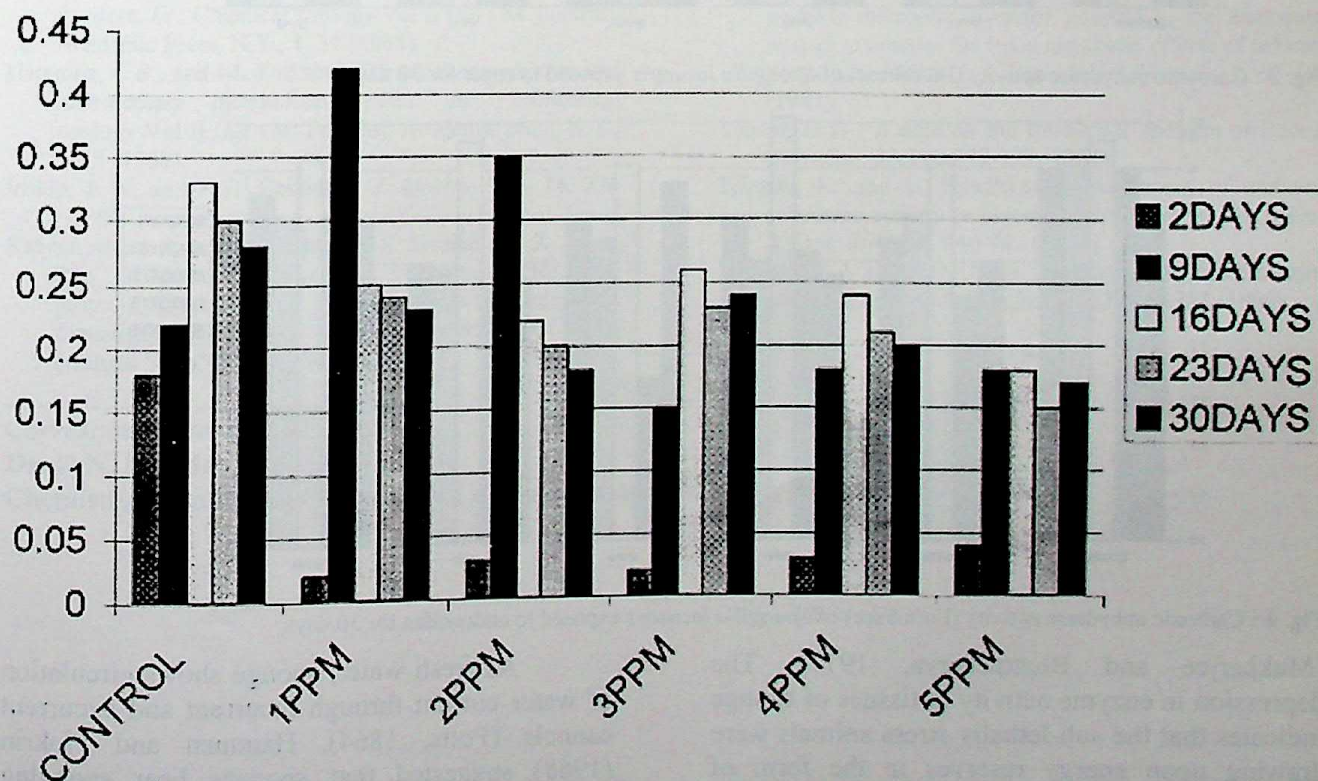
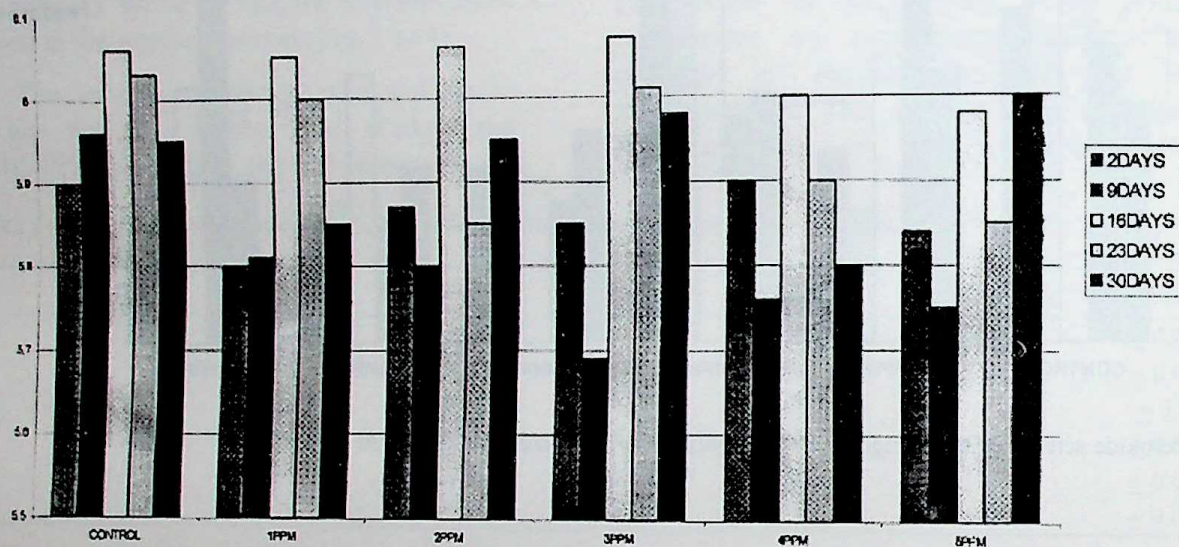
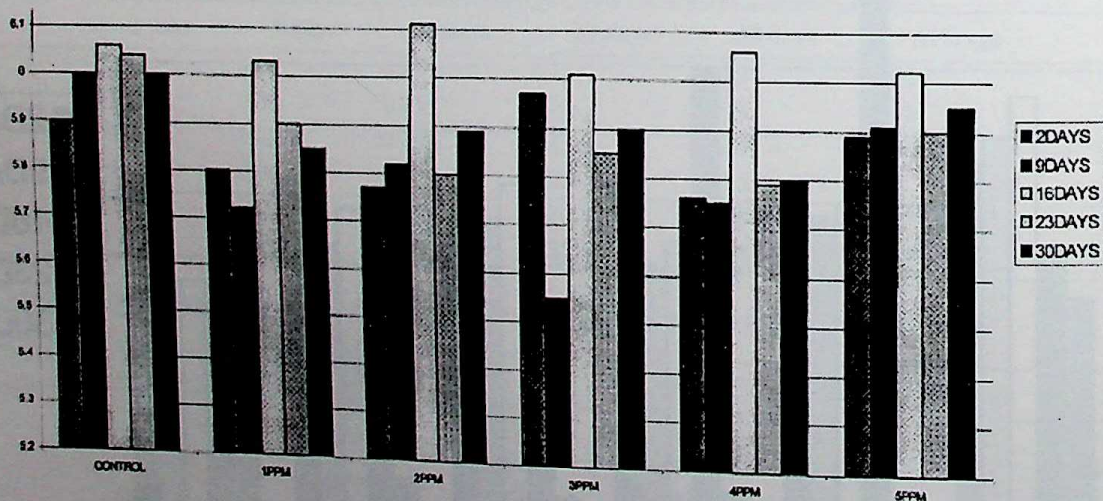


Fig. 2 : Peroxide activity (μ moles/mg/min) of *Spongilla lacustris* exposed to endosulfan for 30 days.

Table-4 : Carbohydrates content (mg/100 mg) of *Spongilla lacustris* exposed to endosulfan for 30 days.

	2 days	9 days	16 days	23 days	30 days	Average	S.D.
Control	0.24	0.3	0.44	0.25	0.23	0.292	0.087
1 ppm	0.29	0.22	0.21	0.18	0.16	0.212	0.049
2 ppm	0.08	0.18	0.15	0.11	0.09	0.122	0.042
3 ppm	0.02	0.4	0.26	0.15	0.12	0.19	0.145
4 ppm	0.03	0.26	0.24	0.16	0.11	0.16	0.094
5 ppm	0.06	0.42	0.22	0.2	0.14	0.208	0.133

**Fig. 3 :** Carbonic anhydrase activity (Eu/ml/sec) of *Spongilla lacustris* exposed to rogar for 30 days.**Fig. 4 :** Carbonic anhydrase activity (Eu/ml/sec) of *Spongilla lacustris* exposed to endosulfan for 30 days.

(Mukherjee and Bhattacharya, 1977). The depression in enzyme activity in tissues of sponge indicates that the sub-lethally stress animals were drawing upon energy reserves in the form of carbohydrates (Dunning and Major, 1974).

As fresh water, sponge shows circulation of water current through incurrent and excurrent canals (Potts, 1864). Hammen and Flokrin (1968) suggested that sponges bear sponging fibres, which are of collagen and collagenic type.

Protein might be helping in the bio-monitoring process including the bio-degradation of insecticides. The decrease in carbohydrate, showed consumption of metabolic energy was higher during particular period of exposure.

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Nickel induced changes on some aspects of protein metabolism in the tissues of *Pila globosa*.

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Abstract : Some aspects of protein metabolism were studied in foot, hepatopancreas and mantle tissues of snail, *Pila globosa* on exposure to lethal concentration for 2 days (336. 7 mg/L) and sublethal concentration (67. 34 mg/L) of nickel for 1, 5 and 10 days. Total, structural and soluble proteins decreased significantly and to continence, this the levels of amino acids and protease activity increased in all the tissues of snail at all time points examined. Activities of AAT (Aspartate aminotransferase) and ALAT (Alanine aminotransferase) showed contrasting trends of inhibition and elevation during lethal and sublethal concentrations of nickel treatment. GDH (Glutamate dehydrogenase) activity was increased in all the tissues with increase in exposure time. Level of ammonia decreased in snails at sublethal concentration, but increment was observed in lethal concentration along with increased urea content. Under lethal and sublethal exposures, the changes in all the parameters were more pronounced in hepatopancreas followed by foot and mantle. At most instances, snails in the lethal medium were affected more compared to sublethal concentration.

Key words : Nickel, Protein metabolism, *Pila globosa*.

Introduction

The concentration of nickel, a VIII B group divalent element has been increasing in the aquatic ecosystems with the release of effluents from petroleum refineries, electroplating, industries manufacturing steel, fertilizers and automobiles in addition to nickel mines (Duke, 1980; Moore and Ramamoorthy, 1984). India is no exception with habitable waters being contaminated with nickel beyond permissible limits (Agadi *et al.*, 1978; Joseph and Srivastava, 1993). Though nickel is essential in trace quantities and plays a vital role in metabolism, at higher concentrations it has deleterious effects (Calabrese *et al.*, 1977a, Eisler and Hennekey, 1977).

Toxic potential of nickel to different aquatic organisms (Khangarot and Ray, 1987; Ali Khan and Stroch, 1990; Ali Khan *et al.*, 1990) including snails (Gupta *et al.*, 1981; Khangarot *et al.*, 1982) is well documented, due to its ability to accumulate in various body tissues (Sadiq and Alam, 1989; Bardeggia and Ali Khan, 1991). Information on nickel induced alterations at

biochemical level (Arillo *et al.*, 1982; Chowdhary and Kedarnath, 1985) and in protein metabolism (Sreedevi *et al.*, 1992) is restricted to only freshwater fishes. The objective of the present study is to delineate the effects of nickel on some aspects of protein metabolism in functionally different tissues of freshwater snail, *Pila globosa* at both lethal and sublethal concentration. This animal model has been selected, as it is suitable for studies involving toxicology (Szeer and Plotr, 1986). Apart, *Pila globosa* occupies a significant position in the food chain of aquatic ecosystems.

Materials and Methods

Freshwater snails, *Pila globosa* weighing 19 ± 2 g were collected from local paddy fields around Dharwad and were stored in glass aquaria in the laboratory at room temperature ($28 \pm 1^\circ\text{C}$). The snails were fed daily with pieces of hydrilla and were adapted to laboratory conditions for 15 days before using them for experimentation. The snails were transferred to plastic tubs and maintained with dechlorinated tap water. The physico-chemical characteristics of the water

were as follows : pH 7.4-7.6 : carbon dioxide 2.08 mg/L : dissolved oxygen 5.7-6.0 mg/L; salinity 0.190 mg/L; alkalinity 102 mg/L as CaCO_3 ; hardness of water 112 mg/L as CaCO_3 ; temperature $28 \pm 1^\circ\text{C}$. Stock solution of nickel chloride was prepared in deionised water and appropriate amounts of toxicant solutions were added to each tub to obtain serial concentration of nickel chloride. The median lethal concentration (LC_{50}) of nickel to the *Pila globosa* was determined by following probit analysis method (Finney, 1964) for 48-hour exposure, and was found to be 336.7 mg/L. For sublethal concentration, one-fifth of the LC_{50} value was selected (67.34mg/L). The snails were divided into 3 groups. The animals of group 1 and 2 were exposed to lethal (336.7 mg/L) and sublethal (67.34mg/L) concentration of nickel respectively. Group 3 served as control. During experimentation period, the animals were fed with hydrilla at a rate of two percent of their body weight, but the feeding intensity of the animal was observed to be very poor. Both the control and experimental (nickel treated) snails were killed after the stipulated time (lethal 2 day; sublethal 1, 5 and 10 day) and the foot, hepatopancreas and mantle tissues were isolated and used for analysis of various biochemical parameters. Each parameter was measured in six individuals of both control and experimental groups.

The protein fractions (total, soluble and structural proteins) were estimated by Folin phenol reagent method (Lowry *et al.*, 1951) after homogenizing the tissues with 10% trichloro acetic acid. Values were expressed as mg/g wet weight of the tissue. Total amino acids were estimated using Ninhydrin reagent (Moore and Stein, 1954) and the values were expressed as mg/g wet weight of the tissue. Ammonia and urea were determined by the methods as described by Bergmeyer (1965) and Natelson (1971) respectively. The values were expressed as μ moles/g wet weight of the tissue.

Protease activity was assayed through the method described by Davis and Smith (1955) and

the activity was expressed as μ moles of amino acids released/mg protein/hr. Aspartate and alanine aminotransferase (AAT and AlAT) were estimated by the method of Reitman and Frankel (1957). The incubation mixture for AAT contained 100 μ moles of phosphate buffer (pH 7.4), 50 μ moles of L-aspartic acid (pH 7.4) and 2 μ moles of L-ketoglutarate. For AlAT the incubation steps followed were as described for AAT, but the substrate used was DL-alanine (20 μ moles). GDH was assayed by the amount of formazan formed/mg protein/hr (Lee and Lardy, 1965). The reaction mixture contained 100 μ moles of phosphate buffer (pH 7.4), 40 μ moles of sodium glutamate, 1.0 μ mole of NAD and 4 μ moles of INT. The mean values of control and nickel exposed snails of all the parameters were subjected to analysis of variance (ANOVA) (Pillai and Sinha, 1968) to determine the significance.

Results and Discussion

Total, structural and soluble proteins (TP, Stp and Sop) in the foot, hepatopancreas and mantle tissues of snail decreased at all the exposure periods of lethal and sublethal concentrations (Table 1). Maximum percent decrease was observed in hepatopancreas followed by foot and mantle. The decrement of all protein fraction were significant except the decrease of Stp in mantle and foot tissues and Sop of mantle tissue after 1 day exposure to sublethal concentration of nickel. The percent decrease observed was more in the lethal concentration compared to sublethal concentration. In support of this, amino acid levels and protease activity increased in all the tissues with the increase in exposure time (Table 1 and 2). The percent increase in amino acids and protease activity were significant in all the tissues at all periods of exposure except the increment after 1 day sublethal treatment of mantle and foot (amino acids) and hepatopancreas and mantle (protease). Significant decrease in AAT and AlAT activities were noticed in all the three tissues of snail exposed to lethal concentration, but increased at

Nickel induced changes in the tissues of Pila globosa.

sublethal concentration (Table 2). The increment in AAT and ALAT in mantle tissue after 1-day exposure was not significant. GDH activity also increased in all the tissues of snail at both lethal and sublethal concentration of nickel treatment

(Table 3). The increment in mantle and foot after 1-day exposure under sublethal concentration was not significant. The remaining values significantly differed from controls. Ammonia and urea levels

Table – 1 : Biochemical changes in different tissues of snail, *Pila globosa* exposed to nickel.

Tissue	Control	Exposure periods in days			
		Lethal (336.7 mg/L)		Sublethal (67 mg/L)	
		2	1	5	10
Total proteins* foot	96.1 ± 4.03	53.8 ^a ± 5.74 (-44.03)	85.5 ^b ± 4.74 (-11.0)	76.4 ^a ± 1.74 (-20.4)	65.9 ^a ± 1.91 (-31.3)
Hepatopancreas	123.2 ± 4.30	61.5 ^a ± 0.19 (-50.0)	98.4 ^a ± 2.70 (-20.1)	83.6 ^a ± 2.40 (-32.2)	66.93 ^a ± 1.91 (-45.6)
Mantle	73.1 ± 2.12	45.5 ^a ± 1.80 (-37.8)	65.3 ^b ± 1.80 (-10.6)	59.5 ^a ± 2.71 (-18.6)	52.4 ^a ± 2.48 (-28.3)
Structural proteins* foot	60.9 ± 3.78	43.8 ^a ± 3.22 (-28.1)	55.9 ^d ± 3.42 (-8.21)	49.7 ^b ± 2.89 (-18.4)	46.6 ^b ± 2.98 (-23.4)
Hepatopancreas	74.5 ± 3.60	45.2 ^a ± 3.10 (-39.3)	59.7 ^a ± 3.81 (-19.8)	53.1 ^a ± 2.98 (-28.6)	50.3 ^a ± 3.17 (-32.4)
Mantle	43.3 ± 3.59	28.5 ^a ± 2.86 (-34.2)	39.9 ^d ± 2.93 (-7.6)	37.8 ^c ± 2.67 (-12.5)	34.1 ^a ± 2.80 (-21.1)
Soluble proteins* foot	36.2 ± 2.46	11.5 ^a ± 2.08 (-68.1)	29.3 ^c ± 3.51 (-19.1)	25.6 ^b ± 2.79 (-29.2)	19.3 ^a ± 2.78 (-46.7)
Hepatopancreas	49.7 ± 3.20	13.8 ^a ± 2.25 (-72.2)	36.1 ^a ± 1.50 (-27.3)	30.4 ^a ± 2.30 (-38.3)	11.04 ± 2.52 (-50.5)
Mantle	29.3 ± 3.51	10.2 ^a ± 2.10 (-55.1)	25.6 ^d ± 2.79 (-12.3)	22.2 ^b ± 2.08 (-24.2)	17.4 ^a ± 2.35 (-40.6)
Free amino acids* foot	1.91 ± 0.07	3.15 ^a ± 0.18 (+64.4)	1.97 ^d ± 0.02 (+3.18)	2.50 ^a ± 0.07 (+30.8)	2.96 ^a ± 0.27 (+54.5)
Hepatopancreas	3.47 ± 0.12	5.93 ^a ± 0.10 (+70.8)	3.78 ^c ± 0.19 (+80.9)	4.90 ^a ± 0.02 (+41.2)	5.72 ^d ± 0.10 (+64.8)
Mantle	1.19 ± 0.07	1.92 ^a ± 0.08 (+61.3)	1.26 ^d ± 0.11 (+5.8)	1.59 ^a ± 0.07 (+33.6)	1.79 ^a ± 0.12 (+50.4)

*mg/g wet weight of the tissue. Data are mean ± SD based on six individual estimations. + and - indicates percent increase and decrease over control. The values were different from corresponding control at ^ap<0.001; ^bp<0.01; ^cp<0.05; ^d not significant.

were also elevated significantly in all the tissues of snails exposed to lethal concentration (Table 3). Under sublethal treatment, ammonia content decreased whereas urea levels increased significantly except in the foot and mantle tissues after 1-day exposure period.

Discussion

Proteins can be expected to be involved in the compensatory mechanisms of stressed organisms (Ramalingam and Ramalingam, 1982;

Sreedevi *et al.*, 1992). The decrease in all protein fractions (TP, Stp and Sop) along with increase in amino acids and protease activity indicate an intensive proteolytic activity in snails exposed to lethal and sublethal nickel concentrations. Decrease in protein content during nickel intoxication suggests an acceleration of protein catabolism and the possibility of impaired protein synthesis. Increased protease activity in the tissues of nickel-exposed snails could be due to lysosomal instability or cellular destruction by high concentrations of the metal (Sternlieb and

Goldfischer, 1976). Increase in free amino acids indicates stepped up proteolysis and fixation of ammonia to keto acids resulting in amino acids formation and these amino acids may contribute to the regulation of ionic balance and to the production of energy during stress (Lowenstein,

1972). The transaminases (AAT and AlAT) serve as strategic link between carbohydrate and protein metabolism under environmental stress (Knox and Greengard, 1965). The steady decrease in AAT

Table - 2 : Biochemical changes in different tissues of snail, *Pila globosa* exposed to nickel.

Tissue	Control	Exposure periods in days			
		Lethal (336.7 mg/L)		Sublethal (67 mg/L)	
		2	1	5	10
Protease* (μ moles of amino acids/mg protein/hr) foot	0.14 ± 0.01	$0.26^a \pm 0.01$ (+81.8)	$0.15^c \pm 0.01$ (+8.39)	$0.19^a \pm 0.01$ (+37.1)	$0.24^a \pm 1.01$ (+69.2)
Hepatopancreas	0.38 ± 0.01	$0.74^a \pm 0.01$ (+91.7)	$0.42^d \pm 0.03$ (+11.4)	$0.55^b \pm 0.07$ (+41.9)	$0.68^a \pm 0.01$ (+76.6)
Mantle	0.11 ± 0.004	$0.19^a \pm 0.01$ (+75.2)	$0.12^d \pm 0.01$ (+3.5)	$0.50^a \pm 0.01$ (+28.3)	$0.19^a \pm 0.01$ (+66.4)
AAT (μ moles of pyruvate formed/mg protein/hr) foot	0.22 ± 0.003	$0.17^a \pm 0.005$ (-22.7)	$0.23^d \pm 0.01$ (+4.50)	$0.25^a \pm 0.003$ (+13.5)	$0.32^a \pm 0.003$ (+45.4)
Hepatopancreas	0.33 ± 0.004	$0.22^a \pm 0.01$ (-36.3)	$0.35^b \pm 0.004$ (+6.0)	$0.47^a \pm 0.01$ (+42.4)	$0.54^a \pm 0.01$ (+63.6)
Mantle	0.25 ± 0.002	$0.19^a \pm 0.01$ (-24.0)	$0.26^b \pm 0.01$ (+3.55)	$0.29^a \pm 0.003$ (+16.0)	$0.38^a \pm 0.004$ (+52.1)
AlAT (μ moles of pyruvate formed/mg protein/hr) foot	0.41 ± 0.04	$0.26^a \pm 0.01$ (-36.5)	$0.47^d \pm 0.03$ (+14.6)	$0.58^a \pm 0.02$ (+41.4)	$0.63^a \pm 0.02$ (+53.6)
Hepatopancreas	1.78 ± 0.02	$0.71^a \pm 0.02$ (-60.1)	$2.14^a \pm 0.02$ (+20.2)	$2.76^a \pm 0.02$ (+55.0)	$3.02^a \pm 0.02$ (+69.6)
Mantle	0.67 ± 0.02	$0.37^a \pm 0.03$ (-44.7)	$0.85^b \pm 0.06$ (+26.8)	$0.99^a \pm 0.01$ (+47.7)	$1.07^a \pm 0.03$ (+59.7)

Data are mean \pm SD based on six individual estimations. + and - indicates percent increase and decrease over control. The values were different from corresponding control at ^a $p < 0.001$; ^b $p < 0.01$; ^c $p < 0.05$; ^d not significant.

activities in the tissues of snails exposed to the lethal concentration of nickel could reflect the suppression of excessively accumulated amino acid transamination (Bhakthavatsalam and Srinivasa Reddy, 1982; Sreedevi *et al.*, 1992). AAT, a key enzyme in nitrogen metabolism and energy mobilization in invertebrates, is often used as biological index of stress (Calabrese *et al.*, 1977b). Increased activities of AAT and AlAT in *Pila globosa* during exposure to sublethal and lethal concentration indicates an active transamination of amino acids which provide keto to serve as precursors in the synthesis of essential

organic constituents under heavy metal stress (Venkatramana and Radhakrishnaiah, 1987). Similar findings by Sreedevi *et al.*, (1992) in fish, *Cyprinus carpio* under lethal and sublethal concentrations of nickel adds support to the present observations. GDH, a mitochondrial enzyme, and catalyses the oxidative deamination of glutamate thus provides ketoglutarate to Kreb's cycle. Increase in GDH activity in the tissues of snails could indicate an induction of this enzyme for the structural recognition of proteins (Kulkarni and Kulkarni, 1987). Increased levels of ammonia in the exposed snails during lethal

Nickel induced changes in the tissues of Pila globosa.

concentration corroborate the increased levels of protein hydrolysis, since ammonia is the main product of protein catabolism (Martin *et al.*, 1983). The decreased ammonia content observed in snails under nickel; stress (sublethal concentration) could be due to conversion of toxic ammonia to less toxic urea (Rajeshwar Rao *et al.*, 1983) as evidenced by the increased levels of urea

in *Pila globosa* after both lethal and sublethal exposures respectively. During stress conditions, animals have the ability to convert highly toxic ammonia to less toxic substance in order to limit the intake of water for ammonia excretion (Ramana Rao and Ramamurthy, 1983). A similar pattern of

Table – 3: Biochemical changes in different tissues of snail, *Pila globosa* exposed to nickel.

Tissue	Control	Exposure periods in days			
		Lethal (336.7 mg/L)		Sublethal (67 mg/L)	
		2	1	5	10
GDH (μ moles of formazan formed mg protein/hr) foot	0.03 ± 0.003	$0.04^d \pm 0.002$ (+8.82)	$0.03^d \pm 0.002$ (+2.94)	$0.04^a \pm 0.003$ (+17.6)	$0.04^a \pm 0.004$ (+26.5)
Hepatopancreas	0.10 ± 0.01	$0.13^c \pm 0.01$ (+24.3)	$0.12^c \pm 0.01$ (+13.6)	$0.15^b \pm 0.01$ (+41.7)	$0.16^a \pm 0.02$ (+58.2)
Mantle	0.05 ± 0.003	$0.06^c \pm 0.003$ (+11.1)	$0.06^d \pm 0.004$ (+7.5)	$0.07^a \pm 0.003$ (+24.1)	$0.07^a \pm 0.003$ (+31.5)
Ammonia (μ moles/g wet wt) foot	2.21 ± 0.04	$2.32^b \pm 0.03$ (+4.90)	$2.10^b \pm 0.03$ (-4.90)	$1.90^b \pm 0.16$ (-14.01)	$1.68^a \pm 0.07$ (-23.8)
Hepatopancreas	3.45 ± 0.09	$3.84^b \pm 0.15$ (+11.3)	$2.93^a \pm 0.03$ (-15.1)	$2.47^a \pm 0.05$ (-28.4)	$1.98^a \pm 0.03$ (-42.6)
Mantle	2.77 ± 0.04	$2.85^c \pm 0.04$ (+2.8)	$2.54^b \pm 0.06$ (-8.3)	$2.31^b \pm 0.03$ (-16.4)	$2.06^a \pm 0.03$ (-25.6)
Urea (μ moles/g wet wt) foot	0.62 ± 0.03	$0.71^c \pm 0.05$ (+14.1)	$0.65^d \pm 0.01$ (+4.17)	$0.75^b \pm 0.05$ (+20.4)	$0.87^a \pm 0.05$ (+39.3)
Hepatopancreas	1.57 ± 0.04	$1.91^a \pm 0.08$ (+21.9)	$1.69^b \pm 0.03$ (+7.7)	$2.02^a \pm 0.03$ (+28.9)	$2.34^a \pm 0.06$ (+51.3)
Mantle	0.82 ± 0.04	$0.92^b \pm 0.01$ (+12.6)	$0.86^d \pm 0.03$ (+5.2)	$1.03^c \pm 0.05$ (+25.2)	$1.16^a \pm 0.06$ (+42.5)

Data are mean \pm SD based on six individual estimations. + and - indicates percent increase and decrease over control. The values were different from corresponding control at ^a $p < 0.001$; ^b $p < 0.01$; ^c $p < 0.05$; ^d not significant.

conversion has been observed in the tissues of the bivalve, *Lamellidens marginalis* (Sreedevi, 1989), and in *Cyprinus carpio* (Sreedevi *et al.*, 1992) exposed to lethal and sublethal concentrations of nickel. In conclusion, the progressive decrease in proteins (total, structural and soluble) and ammonia the increased levels of amino acids, protease, AAT, ALAT, GDH and Urea in the tissues of snails at lethal and sublethal concentrations are manifestations of structural and functional disturbances induced by nickel stress. The separation and characterization of proteins coupled with the qualitative analysis of

amino acids would provide an insight into the exact compensatory role of protein metabolism. Performing such studies at frequent intervals during chronic exposure would enhance the understanding of the biochemical lesions caused under stress conditions.

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A study of neurotoxicity of BHC in relation to residual accumulation on the brain tissue of *Heteropneustes fossilis* (Bloch).

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Abstract : Neurotoxic effect of BHC, the organochlorine pesticide in *Heteropneustes fossilis* has been studied exposing at the dose concentrations of 1 ppm, 5 ppm and 10 ppm in lab aquarium for 96 hours over a period of one year. The results showed the behavioural abnormalities in different exposure concentrations such as dysfunction of endocrine gland, excretion of mucus, depigmentation, sign of restlessness, erratic swimming with rapid jerky movement, spiralling and convulsion showing severe effect in central nervous system. Therefore an attempt has been made for monitoring of BHC residues viz. α , β , γ isomers in the brain tissue exposed to different sublethal concentrations using Gas liquid chromatography. The mean values of isomers were found to be 1.587 $\mu\text{g/gm}$ for 1 ppm, 2.993 $\mu\text{g/gm}$ for 5 ppm and 3.78 $\mu\text{g/gm}$ for 10 ppm test group. Severe behavioural abnormalities were recorded at high dose concentration of pesticides with higher accumulation of pesticide residues in brain tissue.

Key words : Organochlorine pesticide, BHC, Residual accumulation, Behavioural abnormality, Neurotoxicity.

Introduction

Extensive use of organochlorine pesticide to protect the crops in the agricultural fields have resulted serious health hazard to the non-target aquatic organisms mainly fish, which is directly effected by the pesticides due to residue accumulation in their body. Pesticide induced residual effect on fish have been reported by Canton *et al.* (1975), Bakre *et al.* (1990), Khangarot *et al.* (1991), Hazarika and Das (1992).

In the present paper behavioural abnormalities of freshwater catfish *H. fossilis* has been studied along with residual analysis of the brain tissue after short term exposure of organochlorine pesticide benzenhexachloride.

Materials and Methods

Air breathing cat fish *H. fossilis* (body length 8-10 cm, body wt. 20-25grn) were collected from local pond of Barpeta district, Assam and acclimatized in lab conditions in glass aquaria for 15 days. Fish were fed with standard laboratory diet for a week during acclimatization and toxicity test. Commercial grade of hexachlorocyclohexane (Central Insecticides and Fertilizers, Indore, M.P. India) 50% wettable

powder 6.5% γ - (HCH) was used in the study. Acetone : Ethanol (1 : 1 v/v) was used as a solvent for the preparation of stock solution, which was further, diluted to required concentrations of 1 ppm, 5 ppm and 10 ppm in water. Control experiment with carrier solvent was also carried out. Test water were renewed after every 24 hours. Ten fish with five replicates for each test concentration were tested in glass aquaria. Gradual changes in behavioural pattern of fish were observed during 96 hours of exposure to pesticides. The brain of control and experimental fish were removed for pesticide residue analysis to correlate the changes in the behavioural pattern with neurotoxicity resulted due to residue accumulation in the brain tissue. HCH residues were determined by modified method of Mill *et al.* (1963). Standard isomers of BHC were taken from E.P.A., U.S.A. The residue levels were calculated out by measuring the peak height obtained in GLC.

Results and Discussion

The fish exposed to different concentrations of BHC exhibited marked abnormal behaviour such as visible depigmentation alongwith profused mucus

secretion over the entire body. In 1ppm concentration, the toxic symptoms included the visible changes in various physiological activities

marked by frequent opercular movement and gulping of air in earlier period of exposure and reduced in later period. This indicated immediate

Table 1 : Mean \pm SD of HCH isomers and total HCH residuals $\mu\text{g/gm}$ wet weight in brain of *H. fossilis* after 96 hours exposure.

Time of Exposure		96 hours			
HCH Concentration in water		α -HCH	β -HCH	γ -HCH	Total HCH
1 ppm		0.987 \pm 0.24	0.289 \pm 0.15	0.309 \pm 0.085	1.585 \pm 0.475
5 ppm		1.176 \pm 0.15	0.030 \pm 0.022	1.787 \pm 0.714	2.993 \pm 0.886
10 ppm		3.295 \pm 0.428	0.110 \pm 0.05	0.376 \pm 0.079	3.781 \pm 0.557
Control		0.011 \pm 0.007	ND	0.019 \pm 0.004	0.036 \pm 0.011

Data mean of 5 samples; ND=Not Detected.

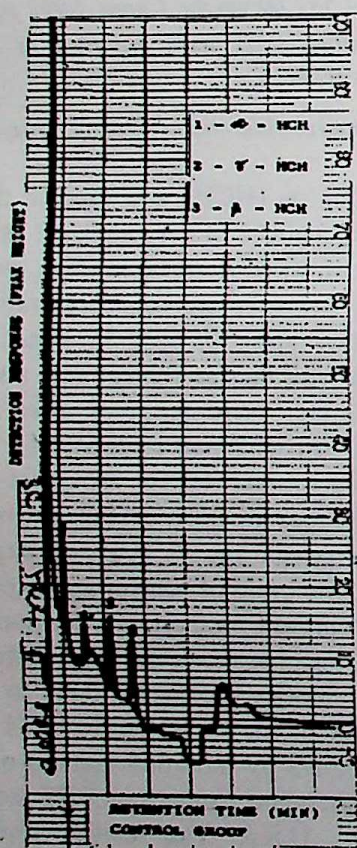


Fig. 1. GL chromatograms of BHC isomers in the control brain extract of *Heteropneustes fossilis*.

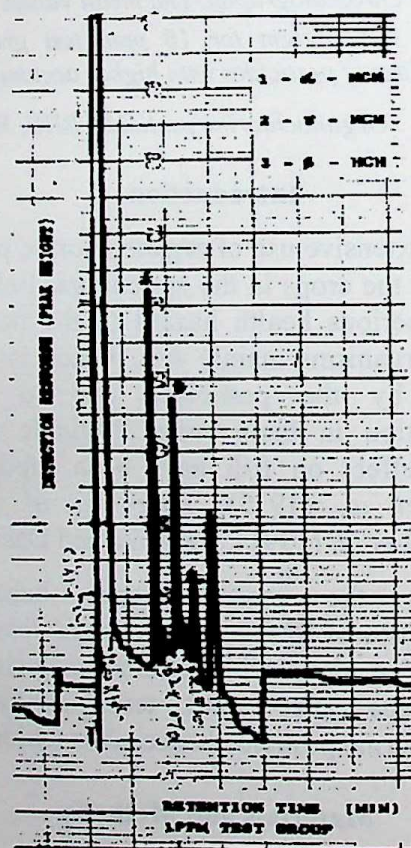


Fig. 2. GL chromatograms of BHC isomers in the brain extract of 1 ppm test group of *Heteropneustes fossilis*.

effect of pesticide in gills caused impairment to respiration. In higher concentration with 5ppm and 10 ppm of BHC, the fish exhibited erratic swimming with rapid Jerky movements, spiralling, convulsion and hyper excitability. Later on fish showed frequent gulping of air with

restricted swimming movement and indicated poor response to the external stimuli. These symptoms followed by disturbed equilibrium and with erratic movement. Fish progressively become lethargic and ultimately sank to the bottom of the aquarium. Food intake was reduced in all the test

Neurotoxicity of BHC to *Heteropneustes fossilis*.

groups, which suggested the effect of pesticides on the normal physiological activities. As it is

expected that abnormal behaviour be due to neurotoxic effect of BHC in the brain tissue, the

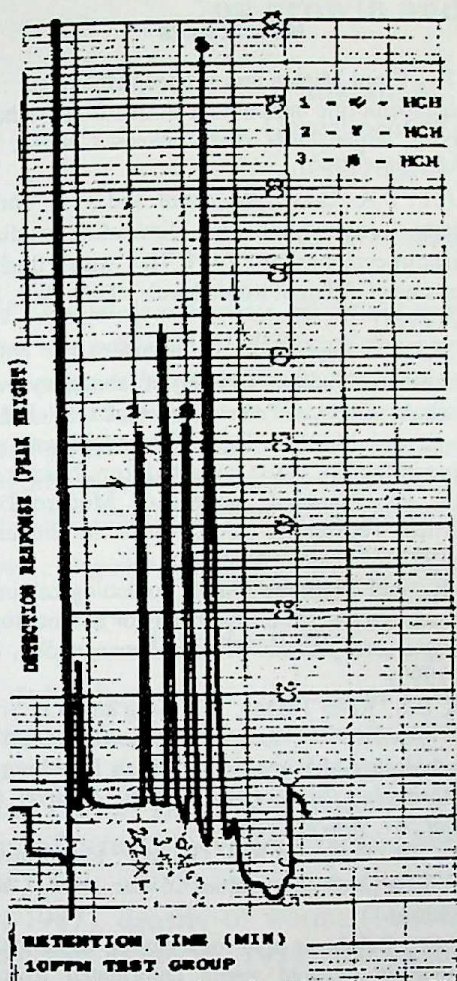


Fig. 3. GL chromatograms of BHC isomers in the brain extract of 10 ppm test group of *Heteropneustes fossilis*.

residual analysis of brain tissue was undertaken. The α , β , γ -HCH residues of brain tissue were measured by Gas Liquid Chromatography. The bioconcentration of total HCH and its isomers in brain tissue of *H. fossilis* are shown in Table 1. The bioconcentration was expressed as a sum of HCH (α -HCH + β -HCH + γ -HCH).

The total residue of HCH ranged from $1.585 \pm 0.475 \mu\text{g/gm}$ to $3.781 \pm 0.557 \mu\text{g/gm}$ in brain tissue in three different concentrations. The degree of accumulation was found to be directly proportional to the dose concentration as bioaccumulation found higher at 10 ppm dose concentration of BHC exposure. Figures 1, 2, 3

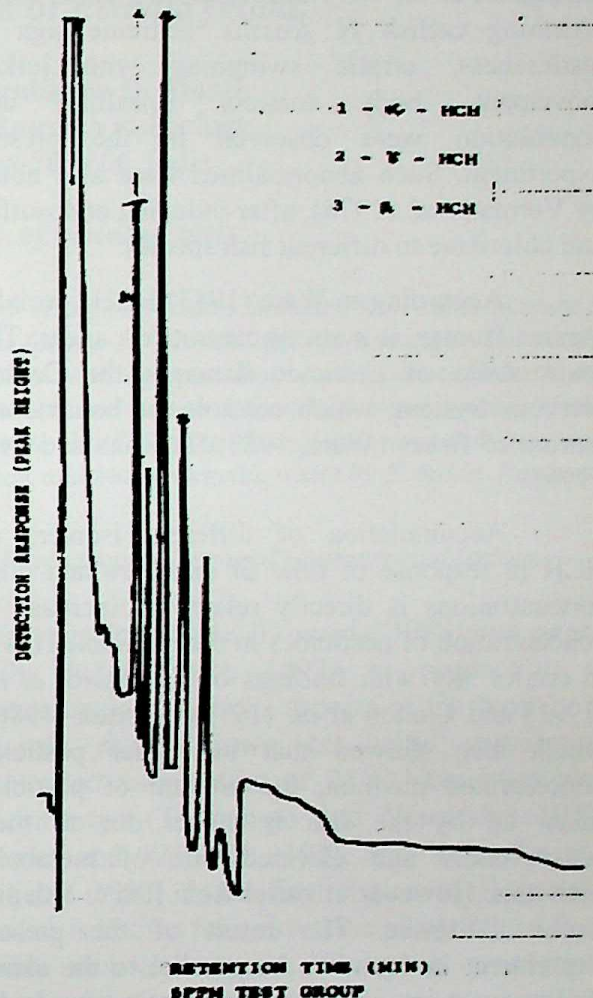


Fig. 4. GL chromatograms of BHC isomers in the brain extract of 5 ppm test group of *Heteropneustes fossilis*.

and 4 represent peak height of BHC isomers in the brain tissue of control, 1 ppm, 5ppm, and 10 ppm dose concentrations.

The residual accumulation of BHC in the brain tissue of experimental fish results in severe dysfunction of central nervous system, which interfere the normal behaviour.

The behavioural abnormalities observed in the present experiment were represented by Verma *et al.* (1984) exposing organochlorine pesticides to Indian fresh water fishes. In this experiment the toxic stress is assumed to be dysfunction an endocrine gland, which results

excretion of mucus and depigmentation along with other behavioural changes was also noted by Khangarot *et al.* 1991 after exposing HCH to air breathing catfish *H. fossilis*. Extreme sign of restlessness, erratic swimming with jerky movement, body torsion, spiralling and convulsion were observed in the present experiment. Such abnormalities were also noted by Verma *et al.* (1978), after inducing endosulfan and chlordane to different fish species.

According to Ware (1983) HCH specially gamma isomer, is a strong neurotoxic agent. The toxic stress of pesticide damages the Central Nervous System, which controls the behavioural pattern of fishes (Ware, 1983; Hazarika and Das, 1999).

Accumulation of different isomers of HCH in response of time of exposure and dose concentrations is directly related to increase in concentration of pesticides in the medium. This is in conformity with findings of Khangarot *et al.* (1991) and Canton *et al.* (1975), Hensen (1980), which also showed that in higher pesticide concentrated medium, the amount of pesticide taken up by the fish is higher due to their sensitiveness and elevated rate of metabolic activities. However, it varies from fish to fish and tissue to tissue. The result of the present experiment is found to be parallel to the above findings, where residue accumulation markedly increases with increasing concentrations. Therefore, it may be concluded that organochlorine pesticide BHC, even in sublethal exposure not only alter normal behaviour and physiological status of the exposed fish, but also get accumulated in the lipid rich brain tissue in a seizable amount and directly effect on Central

Nervous System for its high persistent non-degradable neurotoxic nature.

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Influence of organic wastes and seasonal environmental factors on growth and reproduction of *Eisenia fetida*.

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Abstract : Epigeic earthworms (*E. fetida*) were cultured on variety of organic wastes amended with cattle manure to determine the influence of diets and the seasonal environmental factors on growth and reproduction. The results showed that growth and reproductive strategies of *E. fetida* varied with different diets and seasons. Growth and reproduction of worms in all wastes were significantly more in winter and monsoon than in summer season. Hence winter and monsoon seasons could be considered congenial for vermiculture. During all seasons, worm activities were more in cattle manure followed by amended Bengal gram grain husk and Mixed Organic waste by *E. fetida*. Parthenin containing diet had deleterious effects on cocoon production.

Key words : Organic wastes, Earthworms, *Eisenia fetida*, Growth, Reproduction, Seasonal environmental factors.

Introduction

It has been known that manure worms are very important creatures in the breakdown of organic wastes and release of the nutrients from it (Darwin, 1881). The life activity of a manure worm is influenced by various factors like quality of food (Lee, 1985), moisture (Reinecke and Venter, 1987), density of worms (Nowak, 1975; Reinecke and Reinecke, 1994) and various other abiotic factors (Tsukamoto and Watanabe, 1977; Biradar *et al.*, 1999). Among these, food and the environmental factors play important role on biology of earthworms. Hence, to establish a vermiform in a particular region of the terrestrial ecosystem, it is necessary to know the influence of waste-diets and the environmental conditions prevailing therein.

Many researchers have reported the use of different organic wastes such as sewage sludge (Mitchell *et al.*, 1977; Neuhauser *et al.*, 1988), pig manure (Chan and Griffiths, 1988), pig waste (Wong and Griffiths, 1991), combined sewage sludge and municipal refuse (Grapelli *et al.*, 1983), farmyard-waste (Nardi *et al.*, 1983), cotton industrial waste (Albanell *et al.*, 1988) industrial and vegetable waste (Bano *et al.*, 1987b), animal

and vegetable waste (Edwards, 1988) and paper-mill sludge (Butt, 1993) in production of vermicompost. Various aspects of the biology of *Eisenia fetida* grown on cattle manure at controlled temperature of 25°C have been well documented (Tsukamoto and Watanabe, 1977; Venter and Reinecke, 1988 and Reinecke and Viljeon, 1990). North-East region of Karnataka (India) is known for cultivation of variety of food grains such as redgram, blackgram, greengram, jowar, wheat, rice, bengalgram, and some millets and oil seed crops. Plant residues generated in fields are not properly recycled for nutrient recovery. For converting these organic wastes into valuable biofertilizer (vermicompost) and protein biomass (vermiprotein) it is essential to know the influence of these organic wastes along with prevailing seasonal environmental factors on growth and reproduction of *E. fetida*.

Materials and Methods

Organic wastes used in the experiments were those generated during harvesting [*Cicer arietinum* [Bengalgram grain husk (Bngh)], *Triticum aestivum* [Wheat straw (Whs)], *Oryza sativa* [Rice straw (Riss)], *Dicanthium annulatum* [Grass straw (Grss)], *Sorghum ulgare* [Jowar

straw (Jwrs)], *Cajanus cajana* [Redgram pod husk (Rdph)], *Phaseolus mungo* [Blackgram pod husk (Blph)], *Phaseolus radiatus* [Greengram pod husk (Ggph)], de-weeding {*Parthenium hysteroporus* [Parthenium waste (Prtw)]} and mixed organic waste {[mixture of various agricultural wastes (Mixw)]}. Simultaneously, urine-free cattle manure was also collected and sun dried for use as an adjuvant (for maintaining C : N ratio) of the above wastes.

Agro-based wastes were individually amended with equal amount (v/v) of cattle manure, moistened with tap water to impart 75-80% moisture (Reinecke and Venter, 1987) and stabilized for one week to initiate microbial degradation. Stabilized wastes were individually transferred to fill plastic culture containers (156 cm³ volume and 35 cm² surface area) with perforated lid in triplicates. Cattle manure alone served as control (Graff, 1981) for amended diets. To each container, five one-week aged juveniles of *E. fetida* obtained from stock culture (on cattle manure) were introduced after noting their initial weight. Culture containers were kept in uncontrolled and ventilated room for 16 weeks concomitant with the onset of each season (summer, monsoon and winter). Moisture (75-80%) in culture beds was maintained by daily sprinkling tap water. Scarcity of food was avoided by adding corresponding stabilized organic waste after the original volume in the culture boxes reduced to half.

To findout the influence of seasonal environmental factors on the worms, room temperature (RT) and % relative humidity (%RH) during each season were also recorded every day. The range (mean) of these during summer, monsoon and winter, respectively were 25.00 - 33.82°C (30.49°C) and 34.57 - 55.64% (42.63%), 26.14 - 29.67°C (28.45°C) and 53.78 - 81.71% (67.01%), 24.39 - 30.21°C (26.50°C) and 56.71 - 82.07% (67.46%). Weekly observations in respect of worm biomass, attainment of sexual maturity and cocoon production were noted upto the termination of experiment (16 weeks). The growth rate in mg/d/g live weight was calculated

as detailed by Biradar *et al.* (1999). Cumulative cocoon number/worm was expressed by adding the number of cocoons produced by each worm during 16 weeks. Statistical analyses of the results were carried out with the help of ANOVA test.

Results

Growth and reproductive strategies of *E. fetida* varied with diet and season of the year. All the organic wastes served as source of good diet for the life activities of *E. fetida* during different seasons. Further, cattle manure and amended Bngh and Mixw diets appeared more congenial for this worm over amended pod based and weed wastes. Reproductive activities of the worm in all diets were winter > monsoon > summer season.

Summer Season

Biomass and growth : In summer, biomass and growth rate were more in almost all diets than in other two seasons. Biomass (mean) was maximum in Ggph, followed by cattle manure, Bngh Prtw, Rdph, Mixw, Blph, Jwrs, Riss, Whts and Grss (Table 1). In general, biomass of worms during summer in all waste diets was almost equal to that in winter and more than in monsoon. Increase in weight of worms grown in all diets during summer was gradual during early weeks (1-4 weeks) of their development and later it reached peak (5-7 weeks) upto attainment of maturity. During subsequent weeks (upto 16th week), there was no considerable weight gain. There was no significant ($f = 1.54$) difference in weight of worms among all waste diets (Table 4). The growth rate in all waste diets increased upto maturity of worms and later declined until (16th week) end with significant ($f = 2.83$, $P < 0.001$) variation among various diets. However, no significant difference in growth was noticed between worms grown in cattle manure and other waste diets (Table 4). The maximum ratio between initial weights to growth rate at 16 weeks was (73.15 ± 0.99) in cattle manure and minimum (37.35 ± 0.20) in Whts (Table 1).

Maturity and reproduction : In summer season, all worms grown in different waste diets matured

Influence of organic wastes on the growth and reproduction of E. fetida.

during 6-9 weeks of their development (Table 1). Maturity of all worms as observed by the development of clitellum was attained over two weeks (6-7) duration in Mixw, Riss, Jwrs, while it in cattle manure and Ggph occurred during 7th week. In Grss and Rdph, maturation was much delayed 8-9 weeks and 9th week respectively. Only three worms attained maturity at 7th week in Whts. Cocoon production in most of the diets commenced from 7th week and it was continuous and multimodal in nature. The cumulative (16

weeks) cocoon number (CCN) of each worm was more in cattle manure, Bngh, Mixw and Ggph followed by Rdph, Riss, Blph, Jwrs, Grss, Whts and Prtw (Table 1). There was significant difference among waste diets in respect of CCN ($f = 4.82$, $P < 0.001$) and cocoon production ($f = 3.57$, $P < 0.001$). No significant variation between cattle manure and other waste diets in respect of CCN was observed (Table 4) except between cattle manure & Whts ($f = 2.47$, $P < 0.001$) and cattle manure & Prtw ($f = 2.49$, $P < 0.001$).

Table - 1 : Growth and reproduction of *E. fetida* in cattle manure and amended organic wastes during summer season.

Variables	Cm	Bngh	Mixw	Rdph	Blph	Ggph	Grss	Riss	Jwrs	Whts	Prtw
\bar{X} Biomass (mg)	443.59 ± 65.90	410.27 ± 56.73	322.65 ± 45.66	339.98 ± 57.74	311.04 ± 0.34	445.33 ± 59.47	256.78 ± 38.42	300.33 ± 38.80	303.57 ± 42.41	293.25 ± 37.46	394.37 ± 56.75
\bar{X} Growth rate (mg/d/g)	959.67 ± 109.96	718.57 ± 59.62	651.72 ± 79.77	714.54 ± 90.18	704.22 ± 59.62	1035.18 ± 148.10	553.00 ± 54.50	766.54 ± 104.12	654.07 ± 72.66	517.18 ± 41.48	813.56 ± 83.69
Max. growth rate (mg/d/g) at week	1552.65 ± 23.99 at 7th	1029.04 ± 24.01 at 7th	1154.77 ± 21.98 at 6th	1334.20 ± 72.89 at 9th	955.80 ± 6.84 at 8th	1837.93 ± 30.15 at 7th	830.15 ± 3.79 at 8th	1483.03 ± 31.28 at 5th	1037.36 ± 44.25 at 6th	825.18 ± 1.44 at 5th	1277.99 ± 13.35 at 7th
Maturation at week(s)	7th	7-8th	6-7th	9th	8th	7th	8-9th	6-7th	6-7th	only 3 at 6 th	7-8th
Cumulative cocoon No./ worm at 16th week	19.26 ± 0.30	13.40 ± 0.40	12.80 ± 0.40	9.00 ± 0.40	8.20 ± 0.30	12.06 ± 0.50	4.73 ± 0.30	8.66 ± 0.30	7.66 ± 0.41	4.00 ± 0.11	3.20 ± 0.30
\bar{X} Cocoon rate / worm/ week	1.53 ± 0.20	1.46 ± 0.23	1.30 ± 0.19	1.28 ± 0.09	0.89 ± 0.15	1.20 ± 0.25	0.59 ± 0.18	0.86 ± 0.29	0.76 ± 0.20	0.45 ± 0.06	0.33 ± 0.11
Growth rate ratio at 16th week	73.15 ± 0.99	49.57 ± 1.42	47.57 ± 1.00	55.51 ± 2.94	50.45 ± 0.51	50.64 ± 0.52	54.27 ± 2.59	37.96 ± 0.81	47.82 ± 2.18	37.35 ± 0.20	60.55 ± 0.67

(Data are mean ± SD).

Monsoon Season

Biomass and growth : In this season, biomass and growth rate of worms were less in almost all diets than in other two seasons. Biomass (mean) of worms grown in different waste diets ranged between 240.15 ± 30.36 (in Whts) and 351.89 ± 44.94 (in Bngh) (Table 2). Increase in weight of worms grown in all diets during monsoon was similar to that of summer season.

There was no significant ($f = 0.58$) variation in worm weight among diets and between cattle manure and different waste diets (Table 4). The growth pattern in all diets during monsoon was similar to that in summer season. There was no significant ($f = 0.54$) difference in growth rate of worms among various diets and between cattle manure and other diets (Table 4). The growth rate ratio at 16th week in this season was more than winter and less than summer. The maximum was

(49.28±0.67) in Blph and minimum (35.05±0.28) in Rdph.

Maturity and reproduction : During this season, all worms attained sexual maturity between 5-8 weeks age and started producing cocoons from 6-7 weeks in most of the waste diets (Table 2). Here also, in all diets, the pattern of cocoon production was continuous and multimodal in nature but, it was less than in winter and more than in summer. The cumulative cocoon number (CCN) /worm at 16th week was maximum in cattle manure followed by Bngh, Mixw, Rdph, Riss, Grss, Whts, Ggph, Jwrs, Blph and Prtw (Table 2).

No significant variations were noticed in CCN ($f = 1.24$) and cocoon production ($f = 1.81$)

among different diets and between cattle manure and various diets (Table 4).

Winter Season

Biomass and growth : Biomass of worms grown in different waste diets during winter season did not vary significantly ($f = 1.86$). It in all diets increased rapidly upto 7 weeks and in subsequent weeks (upto 16 weeks), there was no considerable weight gain. Maximum biomass was in cattle manure, followed by Mixw, Rdph, Jwrs, Ggph, Prtw, Blph, Bngh, Riss, Grss and Whts (Table 3). No significant difference was noticed between the biomass of worms grown in cattle manure and different waste diets (Table 4). Similarly, growth rate of worms grown in various diets increased

Table - 2 : Growth and reproduction of *E. fetida* in cattle manure and amended organic wastes during monsoon season.

Variables	Cm	Bngh	Mixw	Rdph	Blph	Ggph	Grss	Riss	Jwrs	Whts	Prtw
\bar{X} Biomass (mg)	268.03 ± 37.76	351.89 ± 44.94	319.44 ± 42.76	267.75 ± 29.11	276.13 ± 42.08	275.38 ± 38.13	269.13 ± 38.42	287.81 ± 36.29	282.77 ± 43.14	240.15 ± 30.36	288.06 ± 38.55
\bar{X} Growth rate (mg/d/g)	563.23 ± 54.26	685.33 ± 63.03	561.57 ± 179.83	577.25 ± 55.49	600.67 ± 63.61	634.27 ± 67.69	570.34 ± 56.15	548.10 ± 48.46	600.24 ± 66.88	585.60 ± 63.03	518.27 ± 43.72
Max. growth rate (mg/d/g) at week	825.48 ± 25.60 at 6th	1032.07 ± 33.74 at 5th	789.06 ± 20.34 at 7th	943.71 ± 10.17 at 5th	932.17 ± 12.38 at 8th	1006.7 ± 48.81 at 6th	876.45 ± 26.91 at 8th	858.09 ± 14.38 at 5th	997.31 ± 8.74 at 8th	982.61 ± 14.71 at 6th	811.78 ± 20.67 at 6th
Maturation at week(s)	6 th	5-7 th	7 th	5-6 th	7-8 th	6-7 th	7-8 th	5-7 th	7-8 th	6-7 th	6-7 th
Cumulative cocoon No./ worm at 16th week	24.46 ± 0.50	21.20 ± 0.40	20.53 ± 0.50	17.33 ± 0.30	13.53 ± 0.50	13.86 ± 0.50	16.06 ± 0.50	16.60 ± 0.40	13.80 ± 0.20	14.06 ± 0.50	9.60 ± 0.20
\bar{X} Cocoon rate / worm/ week	2.22 ± 0.38	1.92 ± 0.30	2.28 ± 0.26	1.57 ± 0.23	1.50 ± 0.21	1.54 ± 0.26	1.60 ± 0.29	1.66 ± 0.26	1.53 ± 0.25	1.40 ± 0.22	0.96 ± 0.19
Growth rate ratio at 16th week	44.56 ± 1.60	43.24 ± 1.73	45.12 ± 1.28	35.05 ± 0.28	49.28 ± 0.67	45.41 ± 2.18	46.06 ± 1.66	39.35 ± 0.44	48.77 ± 0.43	36.82 ± 0.56	37.99 ± 0.98

(Data are means ± SD).

upto maturity and then declined upto the end of 16th week. Growth rate of worms at 6th week was maximum in Mixw (1553.32±19.71) followed by Blph, Cm, Ggph, Rdph, Prtw, Bngh, Riss, Jwrs,

Grss and in Whts (639.39±5.76) (Table 3). There was no significant difference in growth rate of worms between cattle manure and other waste diets; however, significant variation ($f = 4.69$, $P <$

0.001) was noticed among different diets (Table 4). The growth rate ratio at 16th week, among various diets, ranged between 25.5 ± 0.15 (Whts) and 55.85 ± 0.85 (Ggph).

Maturity and reproduction : In all diets, during winter, worms matured during 5 -7 weeks of their development. Sexual maturity in majority of diets (Mixw, Grss, Riss, Whts, Cm, Bngh) occurred at 6th week, while, it in Ggph, Jwrs and Blph though commenced (early) at 5th week in a few, all matured only during 6th week. Maturity occurred late (7th week) in Rdph and Prtw (Table 3).

Cocoon production commenced from 6th week in cattle manure, Bngh, Jwrs, Ggph, Blph; 7th week in Prtw, Mixw, Whts, Riss, Grss and 8th week in Rdph alone. The pattern of cocoon production in winter was similar to that in monsoon and summer seasons. Cocoon production ($f = 4.57$, $P < 0.001$) and CCN ($f = 2.71$, $P < 0.001$) of each worm grown in all diets significantly varied. However, there was no significant variation in cocoon production between cattle manure and other diets

Table - 3 : Growth and reproduction of *E. fetida* in cattle manure and amended organic wastes during winter season.

Variables	Cm	Bngh	Mixw	Rdph	Blph	Ggph	Grss	Riss	Jwrs	Whts	Prtw
\bar{X} Biomass (mg)	438.60 \pm 55.55	304.84 \pm 41.52	386.76 \pm 49.81	338.87 \pm 50.33	306.65 \pm 35.71	327.64 \pm 44.59	234.20 \pm 27.62	299.71 \pm 38.05	332.04 \pm 45.68	221.91 \pm 25.92	318.35 \pm 43.47
\bar{X} Growth rate (mg/d/g)	767.30 \pm 78.61	573.46 \pm 54.24	857.40 \pm 97.98	674.42 \pm 65.46	803.32 \pm 91.40	715.97 \pm 73.07	418.81 \pm 36.93	563.36 \pm 47.64	609.76 \pm 48.60	406.28 \pm 38.87	653.55 \pm 62.96
Max. growth rate (mg/d/g) At week	1286.4 \pm 10.45 at 6th	948.90 \pm 9.01 at 6th	1553.32 \pm 19.71 at 6th	990.72 \pm 45.49 at 8th	1450.26 \pm 12.89 at 5th	1135.97 \pm 13.01 at 5th	648.69 \pm 10.23 at 5th	870.39 \pm 06.99 at 6th	854.51 \pm 14.37 at 5th	639.39 \pm 5.76 at 6th	959.26 \pm 10.90 at 8th
Maturation at week(s)	6 th	6th	6th	7th	5th	5-6th	6th	6th	5-6 th	6th	7th
Cumulative cocoon No./ worm at 16th week	33.06 \pm 0.30	28.33 \pm 0.30	21.26 \pm 0.30	18.20 \pm 0.40	19.33 \pm 0.30	17.40 \pm 0.20	16.6 \pm 0.40	20.20 \pm 0.20	13.40 \pm 0.40	15.33 \pm 0.30	11.80 \pm 0.40
\bar{X} Cocoon rate / worm /week	3.00 \pm 0.31	2.57 \pm 0.32	2.12 \pm 0.20	2.02 \pm 0.41	1.75 \pm 0.26	1.58 \pm 0.23	1.66 \pm 0.16	2.02 \pm 0.20	1.22 \pm 0.18	1.53 \pm 0.12	1.15 \pm 0.28
Growth rate ratio at 16th week	50.46 \pm 0.36	50.37 \pm 0.67	53.05 \pm 0.75	46.13 \pm 2.09	43.13 \pm 0.50	55.85 \pm 0.85	30.06 \pm 0.14	43.34 \pm 0.56	46.48 \pm 0.50	25.51 \pm 0.15	41.43 \pm 0.46

(Data are mean \pm SD).

(Table 4) except that between cattle manure and Jwrs ($f = 2.40$, $P < 0.05$) and Prtw ($f = 2.47$, $P < 0.001$). During this season, cumulative cocoon number of each worm at 16th week (cocoon rate/worm/week) was maximum 33.06 ± 0.30 (3.00 ± 0.31) in cattle manure and minimum 11.80 ± 0.40 (1.15 ± 0.28) in Prtw.

Discussion

Among three seasons, the environmental conditions prevailing during winter in tropical semiarid region were congenial for growth and reproduction of *E. fetida*. Similarly, Amoji *et al.* (2000) reported that winter season was most

suited for culturing *E. fetida* in northeast semiarid climatic region of Karnataka.

The variations in growth and reproduction of *E. fetida* in different diets might be due to its preferential feeding habits (Amoji *et al.*, 1998). Kale and Krishnamoorthy (1981) have also reported that available wastes influence worm activity. The growth pattern of worm was similar upto end, regardless of quality (nutritional status) of diets and seasonal environmental

conditions. This suggested that minimum dietary requirement of the worms was fulfilled by all waste diets used in the experiment. Even in the most favourable (winter) season in different waste diets, there was variation in growth and reproduction of *E. fetida*, which may be attributed to the particle size, texture of raw materials, nutrient status, chemical composition etc. Prosser and Brown (1965) also reported that the influence of organic wastes on

Table - 4 : Seasonal variations ('F' and 'P' values) in biomass, growth, cumulative cocoon number and cocoon production of *E. fetida* in amended organic waste diets from those in cattle manure (control).

		← 'F' values between cattle manure and diets → among diets										f-Value
Seasons		Bngh	Mixw	Rdph	Blph	Ggph	Grss	Riss	Jwrs	Whts	Prtw	
Summer	Worm weight	0.02	0.27	0.20	0.32	0.00	0.64	0.37	0.36	0.41	0.04	1.54
	Growth rate	0.34	0.55	0.35	0.38	0.03	0.96	0.22	0.54	1.14	0.12	2.83**
	Cum. Co. no.	0.69	0.73	0.95	1.29	0.28	1.87	0.55	1.57	2.47**	2.49**	4.82**
	Cocoon rate in cattle manure	0.01	0.06	0.06	0.42	0.12	0.85	0.49	0.64	1.20	1.56	3.57**
Monsoon	Worm weight	0.21	0.29	0.00	0.00	0.01	0.00	0.01	0.01	0.02	0.01	0.58
	Growth rate	0.20	0.00	0.00	0.02	0.07	0.00	0.00	0.02	0.01	0.03	0.54
	Cum. Co. no.	0.01	0.00	0.13	0.33	0.34	0.22	0.14	0.32	0.18	0.66	1.24
	Cocoon rate in cattle manure	0.06	0.00	0.30	0.33	0.30	0.26	0.21	0.31	0.46	1.08	1.81
Winter	Worm weight	0.45	0.07	0.25	0.44	0.31	1.04	0.48	0.28	1.17	0.36	1.86
	Growth rate	0.41	0.30	0.32	0.01	0.03	1.34	0.46	0.27	1.43	0.14	4.69**
	Cum. Co. no.	0.47	0.56	0.89	0.66	1.24	1.2	0.62	1.64	1.24	1.69	2.71**
	Cocoon rate in cattle manure	0.14	0.56	0.65	1.18	1.53	1.29	0.69	2.40*	1.56	2.47**	4.57**

** P = < 0.001, * P = < 0.05

biomass recovery depend upon the factors like texture of materials, nutrient status and chemicals that can act as stimulants or repellents for many invertebrates with respect to food.

Enhanced biological activities of worms cultured in cattle manure (control), Bngh and Mixw in three seasons, as judged by more biomass, growth rate and cocoon production may be due to their quick weathering of wastes resulting into small particle size, release of variety of mineral and organic materials to worm. Bano *et al.* (1987a) have documented the enhanced productivity of *Eudrilus eugeniae* by the addition of additives such as cereals and gram wastes to the cattle dung rather than dung alone.

Earthworms are known (Seenappa and Kale, 1995) to change their behaviour and population structures, when exposed to different substrates.

Increased growth rate of worms during sub-clitellate stage (pre-clitellar stage) in all waste diets during different seasons might be due to the enhanced feeding activities during early weeks of their development. This is in conformity with the observations made by Williams *et al.* (1995) who reported more percent of compost production by *E. fetida* during pre-clitellar than post-clitellar stage. Enhanced biomass and growth rate of worms in all diets during summer than in monsoon and winter was attributed to less cocoon production and consequently the energy needed

for the formation of more cocoons might have been diverted to biomass gain. Fisher and Molnar (1997) have also reported increase in biomass with decrease in cocoon production.

Duration of attainment of sexual maturity of the worms differs with seasons and with diets in the same season. In *E. fetida*, it was between 5-7 weeks in winter, 5-8 weeks in monsoon and 6-9

weeks in summer. The late maturity of worms in summer is attributed to unfavorable seasonal environmental factors [Temperature : $25.00 - 33.82^{\circ}\text{C} = \bar{X} 30.49^{\circ}\text{C}$ and % relative humidity : $34.57 - 55.64\% = \bar{X} 2.63\%$] rather than the nutritional status of diets. Delayed maturity of

Table - 5 : Variations (f) and degree of freedom (DF) in respect of biomass, growth and cocoon production of *E. fetida* grown in amended organic wastes during different seasons

Parameters		Cm	Bngh	Mixw	Rdph	Blph	Ggph	Riss	Grss	Jwrs	Whrs	Prtw
Biomass	F	3.21	1.20	0.68	0.77	0.23	3.26*	0.03	0.25	0.32	1.38	1.36
	DF	47	47	47	47	47	47	47	47	47	47	47
Growth rate	F	5.56**	1.03	3.79*	0.96	1.93	4.23*	2.62	2.76	0.20	3.41*	5.09**
	DF	44	44	44	44	44	44	44	44	44	44	44
Cocoon production	F	3.18	3.56*	5.94**	1.46	3.95*	0.71	5.56**	6.62**	320	13.72**	4.21*
	DF	31	30	28	26	28	29	29	27	29	28	29

** $P < 0.001$, * $P < 0.05$

worms in some diets during same season may be due to the nutritional status of the diet provided. Similar observations have also been reported by Venter and Reinecke (1988) in *E. fetida*. The maturity of worms cultured in different diets during winter and monsoon seasons were in conformity (5-7 weeks) with those at controlled 25°C reported by Venter and Reinecke (1988) and Williams *et al.* (1995).

More cocoon number in Cm (control), Bngh and Mixw during winter than in monsoon and summer might be due to the prevalence of favorable environmental factors for this temperate worm. Further, in Cm, Bngh and Mixw cocoon production was consistently more during all seasons, which might be attributed to small particle size, rich nutrients, palatability and softness of these diets. Nowak (1975) and Lee (1985) have also reported variation in cocoon production by the earthworms with quality of food provided.

In all the seasons, Parthenium waste was the least preferred diet by *E. fetida*. This may be due to the presence of 'Parthenin' content causing

unpalatability of the waste to the worm. Pod based diets (Rdph, Blph, Ggph) though supported worms' life activities could only produce moderate number of cocoons $> \text{Prtw}$ and $< \text{Cm}$, Bngh, and Mixw. It may be due to the presence of, in them, more lignin and phenol contents, which might need more time to degrade and release nutrients. Further, decline in cocoon production in straw-based diets (Whrs, Grss, Riss) might be due to the presence of low nutrients in them and their less palatability.

During favorable season (winter), in most preferred diets (Cm) cocoon production was maximum ($\bar{X} 3.00 \pm 1.00/\text{worm}/\text{week}$) which was more than that reported by Amoji *et al.* (1999) ($\bar{X} 2.60$), Edwards *et al.* (1984) ($\bar{X} 2.5$), Reinecke and Viljeon (1990) ($\bar{X} 2.1$), Neuhauser *et al.* (1980) ($\bar{X} 2.0$) and less than Graff (1982) ($\bar{X} 3.5$). This variation in cocoon production might be due to nutritional status of the diet and uncontrolled seasonal fluctuating environmental factors in our studies.

Seasonal variations : There was no significant difference, between three seasons, in biomass gain by worms grown in all diets (Table 5) except that in Ggph where it was significantly ($f = 3.26$, $P < 0.05$) more. However, growth rate during different seasons were significantly high in worms cultured in Cm ($f = 5.56$, $P < 0.001$) and Prtw ($f = 5.09$, $P < 0.001$) than those in Ggph ($f = 4.23$, $P < 0.05$), Mixw ($f = 3.79$, $P < 0.05$) and Whts ($f = 3.41$, $P < 0.05$). Similarly, cocoon production was also significantly higher in Riss ($f = 5.56$, $P < 0.001$), Grss ($f = 6.62$, $P < 0.001$), Whts ($f = 13.72$, $P < 0.001$), Mixw ($f = 5.94$, $P < 0.001$), than in Blph ($f = 3.95$, $P < 0.05$), Bngh ($f = 3.56$, $P < 0.05$) and Prtw ($f = 4.21$, $P < 0.05$). These variations in biomass and growth rate of worms also might be due to difference in nutritional status of diets and the fluctuations of seasonal environmental factors (temperature and relative humidity) prevailing in this region.

From the above discussion, it could be concluded that growth and reproduction of *E. fetida* in all diets were winter > monsoon > summer seasons. Hence winter and monsoon seasons could be considered more congenial over summer to culture this worm for vermiprotein and vermicompost production. During all seasons, Cm was most preferred diet followed by Bngh and Mixw by *E. fetida*. Parthenium containing diet appeared to have some deleterious effects on *E. fetida*; as was indicated by least number of cocoons.

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Studies on the effect of Isoprocarb (MIPC 50 WP) on livestock.

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Abstract : Different species of livestock was exposed to isoprocarb (MIPC 50 WP) sprays to monitor their health status. Exposure of livestock (calves, sheep, dogs and RIR birds) to 0.1 percent isoprocarb sprays on cotton crop for 6 hours a day for three consecutive days, showed no adverse effects on evident from clinical hematological and biochemical observations.

Key words : Health status, Isoprocarb, Livestock.

Introduction

Pesticides are of much value as crop and grain protectants in agriculture, control of ectoparasites in livestock and insect vectors in public health. Isoprocarb (MIPC 50 WP) a methyl carbamate is marketed in India as an insecticide. It is commonly recommended as a crop protectant specially against the sucking pests of cotton, rice hoopers and aphids on safflower (Murthy *et al.*, 1990; Haung and Pang, 1992; Yang Limei *et al.*, 1995). Livestock are exposed to pesticide sprays when they graze near or near the crop fields (Maddy, 1977). Exposure of livestock to pesticides can cause a variety of subtle irreversible adverse effects in man and animals (Chauhan, 1988). The toxicological investigations of isoprocarb under Indian conditions are inadequate. As per the regulations laid down in the Indian Insecticides Act (Asia Law House, 1968) animal toxicity data must be generated under local conditions for any new pesticide to be used in pest control (Gaitonde, 1978). Therefore, the present study was undertaken to monitor the health status of different species of livestock exposed to isoprocarb under field conditions.

Materials and Methods

The test sample of the insecticide formulation used was Hexamycin 50 WP (M/S Bharat Pulverising Mills Limited, Mumbai).

A field exposure trial to assess health status of livestock exposed to isoprocarb sprays in the field was carried out as per the protocol laid out in "Health Monitoring Survey of Exposure of Men and Livestock to Carbamate Pesticide Sprays in Agriculture", as approved by the Central Insecticides Board, Government of India, Faridabad. The observations were recorded three days as pre-exposure, three days as exposure and seven days post-exposure period. The details of spray schedules are given in Table 1. During exposure period, the animals were kept in the cotton fields, where the isoprocarb sprays were carried out for 6 h per day.

Thirty-two animals were selected to assess the adverse effects, if any, of exposure to isoprocarb sprays. Four (two males and two females) animals of each species viz. calves, sheep and dogs were secured with long ropes, to enable their free mobility in the spray fields. Four WLH birds were kept in cages in the spray fields. Similarly, four animals of each species were exposed to blank formulation sprays as unexposed controls.

The detailed clinical, hematological and biochemical observations were recorded as per the 'Protocol'. The hematological estimations were carried out as per the standard methods (Schalm, 1965; Archer and Jefcott, 1977). The blood samples were collected from each animal at

the same time daily during the pre-exposure and exposure periods and on the third and seventh day after exposure period for hematological and biochemical analysis such as total RBC count (Neubar's chamber), total WBC count (Neubar's chamber), hemoglobin (acid hematin method), erythrocytic sedimentation rate (ESR) (Wintrobe method) and differential leucocyte count (DLC)

(Wright's stain). Blood cholinesterase (Robbins *et al.*, 1958), blood plasma urea nitrogen (Diacetylmonoxime method), serum total protein (Biuret method), serum albumin (BCG dye binding method), serum glutamic oxaloacetate transaminase (SGOT), and serum glutamic pyruvate transaminase (SGPT) (Reitman and Frankel method) and serum alkaline phosphatase

Table - 1 : Schedule of isoprocarb (MIPC 50 WP) sprays on cotton field.

Particulars	Days of spray operation		
	First	Second	Third
Area field covered	1.2	1.2	1.2
Spray volume (L)	600	600	600
Isoprocarb concentration (% a.i.)	0.1	0.1	0.1
Quantity of a.i. utilized (kg)	0.6	0.6	0.6

Table - 2 : Hematological changes in livestock exposed to isoprocarb (MIPC 50 WP) field sprays.

Parameter	Period	Species			
		Calves	Sheep	Dog	Poultry
Total red blood cell counts (millions / cu mm)	Pre-exposure	7.08 ± 0.78	9.25 ± 0.52	6.68 ± 0.73	2.56 ± 0.32
	Post-exposure	7.61 ± 0.70	9.18 ± 0.46	6.81 ± 0.60	2.64 ± 0.30
White blood cell counts (thousands / cu mm)	Pre-exposure	7.45 ± 1.00	8.53 ± 1.04	11.76 ± 0.65	22.44 ± 1.76
	Post-exposure	7.76 ± 1.39	9.26 ± 0.79	12.16 ± 1.09	23.08 ± 2.00
Hemoglobin level (g %)	Pre-exposure	7.45 ± 0.65	7.55 ± 0.64	13.5 ± 0.94	8.55 ± 0.78
	Post-exposure	7.4 ± 0.75	7.75 ± 0.63	13.5 ± 1.04	8.5 ± 0.83
ESR (mm/hr)	Pre-exposure	—*	—*	10.75 ± 1.63	7.5 ± 1.82
	Post-exposure	—*	—*	9.75 ± 1.67	8.25 ± 1.98
Neutrophill counts (%)	Pre-exposure	30.25 ± 4.20	36.75 ± 4.46	65.25 ± 2.16	31.25 ± 2.43
	Post-exposure	30.5 ± 3.72	35 ± 3.66	67.75 ± 2.30	31.75 ± 1.92
Lymphocyte counts (%)	Pre-exposure	56.75 ± 3.25	54.25 ± 4.56	20.5 ± 2.70	55.75 ± 2.07
	Post-exposure	53.75 ± 1.56	54.25 ± 3.05	21.75 ± 2.68	54.5 ± 1.48
Eosinophill counts (%)	Pre-exposure	12 ± 2.35	5.5 ± 0.83	7.75 ± 0.74	1.75 ± 0.22
	Post-exposure	11.5 ± 2.51	4 ± 0.50	6.75 ± 1.80	2.25 ± 0.41
Monocyte counts (%)	Pre-exposure	3.5 ± 0.43	2.5 ± 0.43	6.5 ± 0.56	9.25 ± 0.82
	Post-exposure	4.25 ± 0.06	2 ± 0.36	6.25 ± 0.96	9.25 ± 0.41
Basophill counts (%)	Pre-exposure	—*	—*	—*	2 ± 0.35
	Post-exposure	—*	—*	—*	2.25 ± 0.41

*The ESR in calves and sheep was not recorded.

**The basophill counts in calves, sheep and dogs ranged between 0 and 1%.

The differences between the pre-exposure and post-exposure values of all the parameters were statistically insignificant.

Table - 3 : Biochemical profile of livestock exposed to isoprocarb (MIPC 50 WP) field sprays.

Parameter	Period	Species			
		Calves	Sheep	Dog	Poultry
Blood cholinesterase activity (micromoles of Achhydrolysed/ml at 37°C for 30 min)	Pre-exposure	0.797 ± 0.011	0.696 ± 0.017	0.745 ± 0.018	0.58 ± 0.019
	Post-exposure	0.805 ± 0.006	0.695 ± 0.022	0.748 ± 0.026	529 ± 0.015
Serum total protein (g/dl)	Pre-exposure	7.22 ± 0.71	7.44 ± 0.87	6.5 ± 0.88	4.75 ± 0.28
	Post-exposure	7.3 ± 0.66	7.49 ± 0.86	6.54 ± 0.94	5.13 ± 0.49
Serum albumin (g/dl)	Pre-exposure	3.16 ± 0.32	2.77 ± 0.39	2.37 ± 0.30	1.54 ± 0.30
	Post-exposure	3.19 ± 0.38	2.56 ± 0.27	2.37 ± 0.28	1.52 ± 0.28
Blood plasma urea nitrogen (mg/dl)	Pre-exposure	32.4 ± 6.45	29.22 ± 5.25	26.25 ± 3.83	30.6 ± 5.37
	Post-exposure	31.95 ± 5.26	32.22 ± 5.71	25.35 ± 4.19	32.5 ± 4.71
Serum GOT (units/ml)	Pre-exposure	75.5 ± 23.68	69 ± 17.34	53.5 ± 12.55	120.5 ± 15.56
	Post-exposure	81 ± 13.80	68.5 ± 13.80	58 ± 12.20	100.5 ± 12.75
Serum GPT (units/ml)	Pre-exposure	29.75 ± 9.09	30.25 ± 5.54	44.75 ± 17.59	0
	Post-exposure	34.75 ± 13.92	34 ± 2.38	49.5 ± 12.11	0
SAP (K.A. units)	Pre-exposure	51.15 ± 13.51	66.22 ± 6.36	53.1 ± 15.25	29.7 ± 6.90
	Post-exposure	52 ± 13.43	66.82 ± 6.47	49.82 ± 12.72	31.82 ± 8.69

The differences between the pre-exposure and post-exposure values of all the parameters were significantly insignificant.

(Kind and King's method) were also measured. The treatment effects were compared by 't' test (Sheth *et al.*, 1972) to find out the differences, if any, between the pre-treatment and post-treatment values.

Results and Discussion

The results of various hematological and biochemical estimations among all the species of livestock exposed to field sprays are summarised in Table 2 and 3.

All the animals and birds exposed to isoprocarb field sprays did not show any adverse clinical manifestation or change in their behavior during the exposure period, as well as during the post-exposure period, indicating that no harmful effects of isoprocarb sprays for 6 h on three consecutive days.

The detailed hematological examination of different species of livestock did not reveal any significant change in the cell counts (red blood cells, white blood cells and DLC), hemoglobin and erythrocyte sedimentation rate (ESR). The

ESR in calves and sheep was not recorded, as it has no clinical significance due to extremely slow sedimentation rate (Schalm, 1965; Archer and Jeffcott, 1977).

The biochemical profile of livestock exposed to isoprocarb sprays in cotton fields did not reveal any significant change in whole blood cholinesterase, serum total protein, serum albumin, plasma urea nitrogen, SGPT, SGOT and SAP during post-exposure periods as compared to the respective pre-exposure values. The blood ChE activity livestock is almost due to the AchE in RBC; hence, its activity in whole blood was measured for convenience (Robbins *et al.*, 1958). The mean blood ChE activity of the livestock exposed to isoprocarb sprays during the post exposure period was almost identical to the pre-exposure period. From the present study, it is concluded that the exposure to 0.1 percent sprays of isoprocarb had no impact on the health of livestock as evident from clinical, hematological and biochemical observations.

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Physico-chemical characteristics of the Vellar estuary in relation to shrimp farming.

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Abstract : All the physico-chemical parameters such as temperature, LEC, salinity, pH, dissolved oxygen and nutrients like total phosphorus, inorganic phosphate, nitrite and silicate studied in relation to shrimp farming. There are as many as 42 shrimp farms situated on the banks of Vellar estuary. These farms discharge the used water into the estuary, which may influence the biota there. In the present study the physico - chemical feature in relation to shrimp farming were studied in 3 stations of the estuary. When compared with the previous data from Vellar estuary there was no much difference in physico-chemical characteristics due to shrimp farming.

Key words : Physico-chemical characteristics, Shrimp farms, Vellar estuary.

Introduction

Shrimp farming picked up fast in India particularly in the southern states, due to promotion on the part of Government agencies. The discharge of used water from the farms into the estuaries, backwaters and mangroves became a matter of great concern of the environmentalist. In the absence of reliable data collected over a period, no definite conclusion could be drawn about the negative impact of shrimp farming. This emphasized the need for monitoring different physico-chemical and biological parameters of the estuaries, backwaters and mangroves where shrimp farming has a strong presence. Shrimp farms started their operation in and around Vellar estuary (Lat. 11° 29' N; 79° 46' E) in the year 1992 and by 1995, there were as many as 42 shrimp farms with water spread area of 150 ha. The formulated shrimp feed pellets used are rich in protein, fat, fiber, minerals etc. These nutrients are discharged into the Vellar estuary during periodic draining. Though numerous studies have been carried out in the Vellar estuary, this aspect has not been probed. Therefore, in the present study physico-chemical parameters were collected for a period of 2 years with the view of ascertaining changes in their levels arising out of

draining of used water from shrimp farms into the estuary.

Materials and Methods

Samples of water were collected at monthly intervals from three stations (Fig.1) for a period of two years (1996-1997). Temperature was measured using a standard Celsius thermometer. Light penetration was determined using a secchi disc and the extinction coefficient (K) was calculated using Pool and Atkins (1929) formula. Salinity was estimated with the help of a salinometer model E-2 and pH was measuring using a Elico pH meter. Dissolved oxygen and nutrients were determined using standard methods (Strickland and Parsons, 1972).

Results and Discussion

Monthly variations and mean values of temperature, LEC, salinity, pH, DO and nutrients are given in Table 1.

Temperature : Seasonal variations were observed in atmospheric temperature (Fig. 2) and water temperature (Fig. 3) showed distinct bimodal oscillations at all the three stations. Maximum temperature was noticed during summer season due to dry weather condition. The

minimum temperature was recorded; monsoon could be ascribed to the effect of atmospheric cooling. In the present study temperature showed that atmospheric variation including insolation play major role governing temperature and water exchange between the sea and the estuary is of less significance. Similar conclusion has also

been drawn from this estuary (Chandran and Ramamoorthy, 1984).

Light extinction co-efficient (k) : The distinct variations were observed in LEC (Fig. 4). It was high during the monsoon seasons at all the three

Table - 1 : The mean values and ranges of physico-chemical parameters of stations 1, 2 and 3.

Parameters	Year	Station 1			Station 2			Station 3		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Air temperature ($^{\circ}\text{C}$)	1996	27.2	35.2	31.5	26.1	35.4	31.4	27.7	35.7	32.0
	1997	28.0	35.1	32.04	27.8	35.2	32.11	28.1	35.2	32.4
Surface water temperature ($^{\circ}\text{C}$)	1996	23.0	34.5	29.5	23.5	34.5	29.7	23.0	35.0	29.7
	1997	23.5	33.5	28.8	23.5	34.0	29.0	23.0	34.5	29.0
pH	1996	7.4	8.5	7.9	7.4	8.4	7.8	7.4	8.1	7.7
	1997	7.3	8.4	8.0	7.1	8.3	7.9	7.1	8.1	7.8
LEC (k)	1996	2.2	8.9	5.1	2.6	8.9	5.2	2.3	2.4	5.3
	1997	2.3	9.0	5.3	2.4	9.1	5.4	9.0	9.3	5.5
Salinity (‰)	1996	6.0	36.0	27.9	6.0	36.0	26.3	4.0	34.0	23.3
	1997	4.0	36.0	26.9	4.0	36.0	25.2	0	35	24.0
DO (ml l^{-1})	1996	4.3	7.4	6.4	4.2	7.3	6.2	4.0	7.4	6.1
	1997	4.5	7.5	6.6	4.2	7.3	6.4	4.0	7.3	6.2
Total phosphorus (μM)	1996	0.34	2.17	0.94	0.47	2.12	0.93	0.38	2.15	0.93
	1997	0.24	2.23	0.92	0.19	2.21	0.84	0.23	2.19	0.88
Inorganic phosphate (μM)	1996	0.13	1.78	0.60	0.09	1.74	0.59	0.17	1.89	0.61
	1997	0.16	1.83	0.66	0.10	1.77	0.60	0.16	1.87	0.57
Nitrite (μM)	1996	0.13	2.32	1.02	0.10	2.30	0.97	0.09	2.40	0.98
	1997	0.08	2.34	0.84	0.08	2.31	0.81	0.06	2.34	0.80
Reactive silicate (μM)	1996	0.90	49.30	16.32	0.70	37.10	13.77	1.10	51.30	18.62
	1997	0.06	44.2	14.05	0.09	39.90	12.83	0.18	49.73	13.34

stations due to the low intensity of solar radiation and higher concentration of dissolved organic matter and churning of bottom sediments. Further inundation by land runoff, discharges from shrimp farms, wave action, wind action, fresh water discharge factors in governing light penetration. The turbidity of water column gradually increased from summer to monsoon season.

Salinity : Annual cycle of salinity showed unimodal oscillation (Fig. 5). Salinity regimes was high at all 3 stations during summer season which was due to low rainfall, decreased fresh

water inflow, land drainage and rise in temperature in the estuary owing to its shallow nature leading to consequent evaporation. During monsoon period may due to be characterized by zero salinity indicating that neritic water greatly replaced the fresh water at stations 3. Further, it is evidenced by negative correlation ($r=-0.80$ at station 1, $r=-0.80$ at station 2 and $r=-0.78$ at station 3) obtaining between salinity and rainfall. Salinity data of the present study that two extreme condition one in during summer due to marine dominance and fresh water dominance in monsoon.

Shrimp farming and physico-chemical characters of estuary.

pH : The monthly variation of pH did not show any remarkable variations (Fig. 6). pH was higher during the summer season due to high photosynthesis organisms (Subramanian and Mahadevan, 1999). The low pH was during

monsoon season due to the influence of fresh water influx, dilution of seawater, low temperature and organic matter decompositions (Zingde *et al.*,

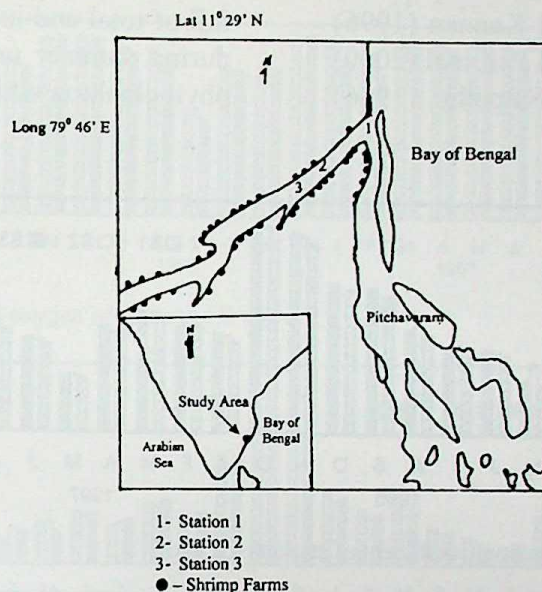


Fig. 1 : Map showing the study area and location of shrimp farms.

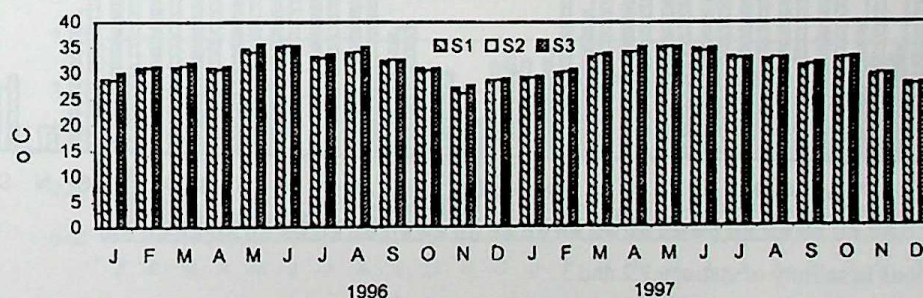


Fig. 2 : Monthly variations in atmospheric temperature of stations 1, 2 and 3.

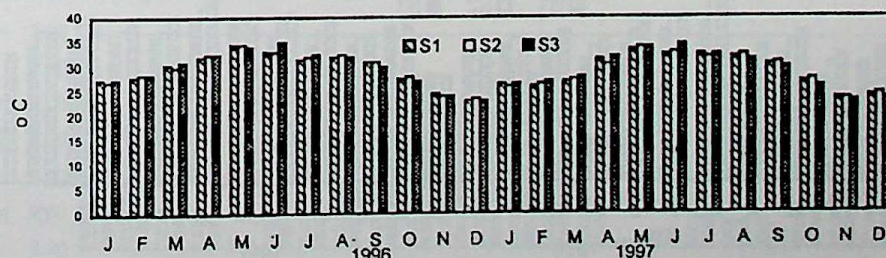


Fig. 3 : Monthly variations in surface water temperature of stations 1, 2 and 3.

1987). Such types of variation in pH values have also been reported from Vellar estuary (Chandran and Ramamoorthy, 1984) and Uppanar estuary (Murugan and Ayyakkannu, 1991).

Dissolved oxygen : DO concentration showed bimodal oscillations (Fig. 7). Higher DO values were obtained during summer and monsoon seasons. During summer seasons due to

phytoplankton photosynthesis, which acted as the major factor influencing the oxygen, distribution and monsoon could be reason for higher solubility in low salinity and low water temperature. Similar type of temperature, pH, salinity and DO was observed by Chandran and Ramamoorthy (1984) from Vellar estuary, Kannan and Kannan (1996) from Palk Bay, Karuppasamy and Perumal (2000) from Pichawaram waters and Satpathy (1996) from Kalpakkam coastal waters.

Nutrients : The monthly phosphate showed distinct variations. Higher concentration of total phosphorus (Fig. 8) and inorganic phosphate (Fig. 9) were noticed during monsoon season which was due to heavy rainfall, land runoff and nutrients enriched shrimp farms discharge. Rapid fall of total and inorganic phosphate were noticed during summer may be due to its utilization by phytoplankton which was high density in summer.

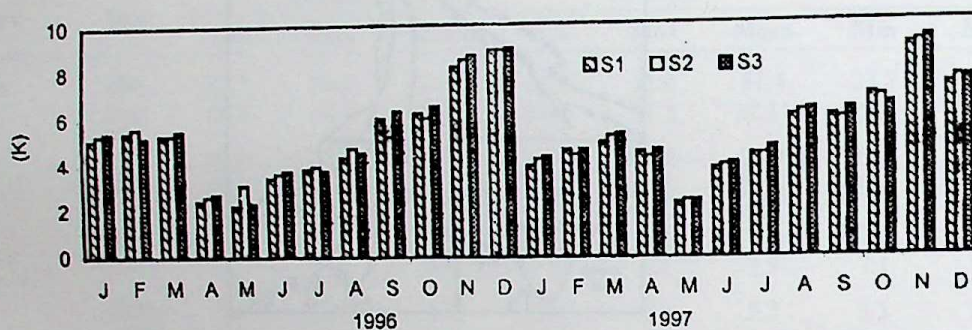


Fig. 4 : Monthly variations in Light Extinction Coefficient of stations 1,2 and 3.

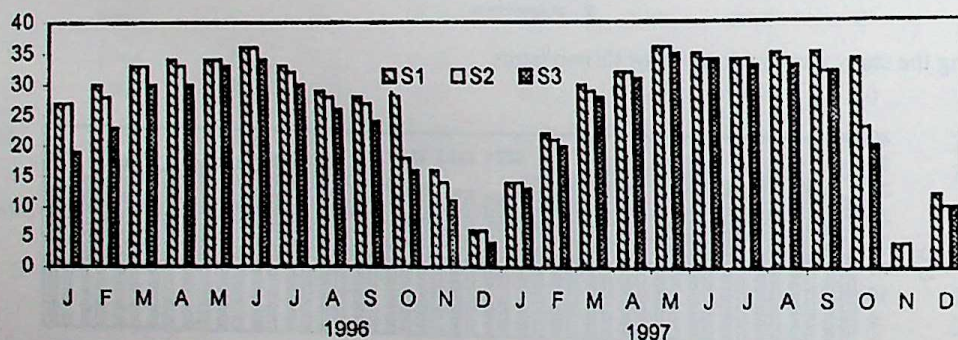


Fig. 5 : Monthly variations in salinity of stations 1,2 and 3.

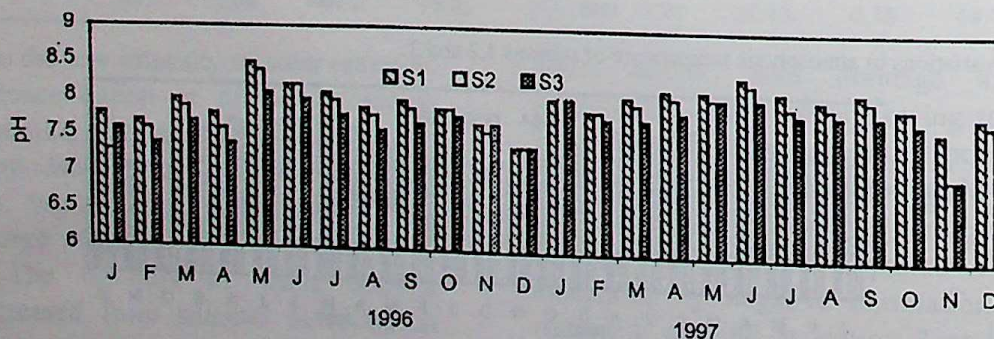


Fig. 6 : Monthly variations in pH of stations 1,2 and 3.

Similar trend was noticed in various estuaries (Chandran and Ramamoorthy, 1984; Murugan and Ayyakkannu, 1991). The variation may be due to the various processes like adsorption and

desorption of phosphate and buffering action of sediment under varying environmental condition (Pomeroy *et al.*, 1965).

Seasonal variation of nitrite at all 3 stations depicted variable concentrations (Fig. 10). Higher concentration was recorded during monsoon, which could be attributed to the variation in phytoplankton excretion, oxidation of

ammonia and reduction of nitrate (Kannan and Kannan, 1996). Similar type of nutrients was observed by Satpathy (1996) from coastal waters of Kalpakkam, Sujatha Mishra and Panigrahy (1993) from Bahuda estuary. Choudhury and

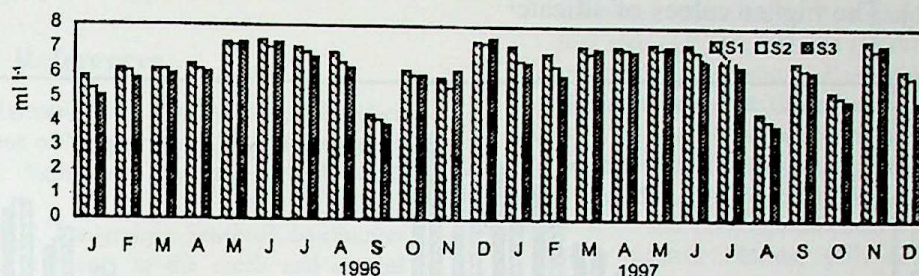


Fig. 7 : Monthly variations in dissolved oxygen of stations 1,2 and 3.

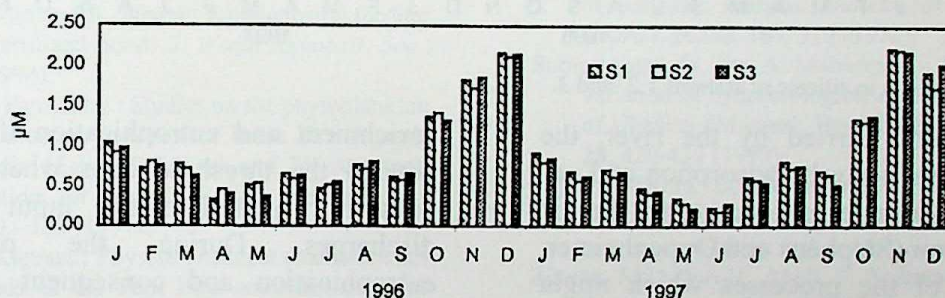


Fig. 8 : Monthly variations in Total phosphorus of stations 1,2 and 3.

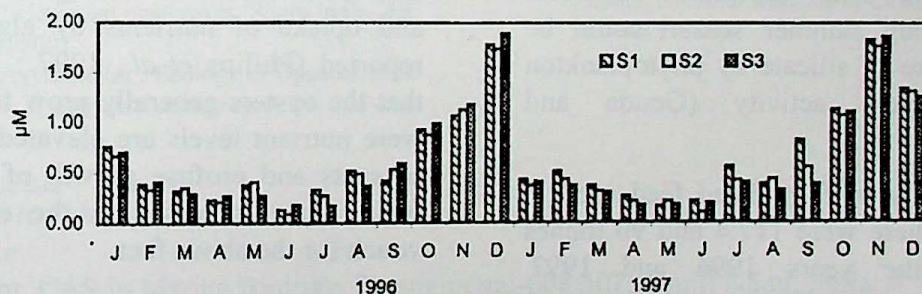


Fig. 9 : Monthly variations in inorganic phosphorus of stations 1,2 and 3.

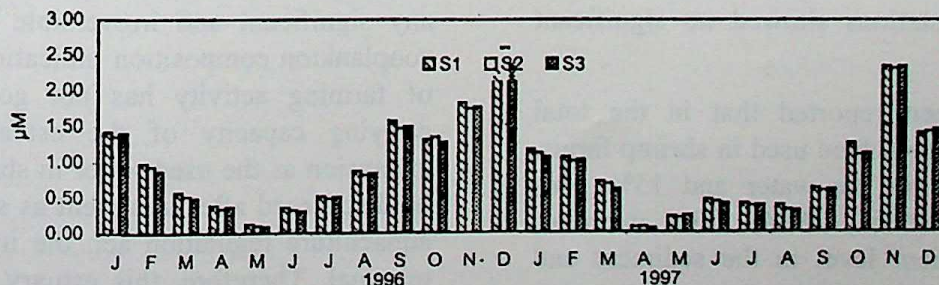


Fig. 10 : Monthly variations in nitrite of stations 1,2 and 3.

Panigrahy (1991) as stated that the distribution and behaviour of nutrient in the coastal environment particularly in the near shore water

and estuaries exhibit considerable seasonal variation depending upon the local condition like rainfall, quantum of fresh water inflow, tidal

incursion and some biological activity like phytoplankton uptake and regeneration. The present study was observed similar trend in this estuary.

Silicate showed conspicuous seasonal variations (Fig. 11). The higher values of silicate

were recorded during monsoon season was due to the addition of silica material by land runoff caused by flooding and also silicate from the bottom sediment might have been exchanged with the overlying water due to the turbulent nature of water in this estuary. Besides this, the dissolution

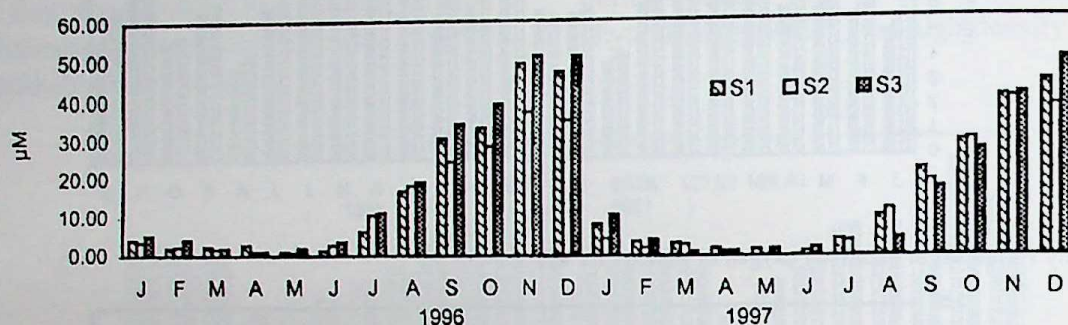


Fig. 11 : Monthly variations in silicate of stations 1,2 and 3.

of particulate silicon carried by the river, the removal of soluble silicate by adsorption and co-precipitation of soluble silicon with humic compounds and iron (Stephens and Oppenheimer, 1972) are some of the processes which might have caused the depletion of silicate when the river water mixes with seawater. The low concentration during summer season could be attributed to uptake of silicate by phytoplankton for their biological activity (Gouda and Panigrahy, 1991).

The quantity of formulated feed used in the shrimp farms here were 117.4 and 90 tonnes respectively in the years 1996 and 1997. However, the inter annual variations in the 3 zooplankton composition tested through the t-test in all the three stations showed no significant differences.

It has been reported that in the total quantity of formulated feed used in shrimp farms, 10% is dissolved in the water and 15% goes uneaten (Diana *et al.*, 1994). The microbes increase the nutrient level in the sediment and water by decomposing this uneaten component. When the water from shrimp farms are discharged in to the natural bodies of water during regular water exchange and along with sediments during harvest at the end, there are chances for nutrient

enrichment and eutrophication if the levels are beyond the threshold ones when tidal flushing does not neutralize the input through farm discharges. During the present study, eutrophication and consequent mass mortality were not observed in the estuarine environment. The lower values are attributed to tidal flushing and uptake of nutrients by algae. It has been reported (Philips *et al.*, 1997 : Sakthivel, 1998) that the oysters generally grow faster in estuaries where nutrient levels are elevated. The settlement of spats and profuse growth of oyster colonies, which have increased in the estuary, recently, vouch for the above fact.

The present study clearly showed that the discharges from the shrimp farms did not cause any significant and irreversible changes in the zooplankton composition indicating that the level of farming activity has not gone beyond the carrying capacity of the estuary. Even with expansion as the used water in shrimp farms is to be discharged after treatment as stipulated by the aquaculture regulation act, the influence will be minimal. Therefore, this estuary can be utilized for shrimp farming up to the carrying capacity.

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Role of probiotics on the environment of shrimp pond.

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Abstract : Recent disease outbreak in shrimp farming caused mainly by bacteria, virus, fungi or a combination of these etiologic agents is attributed to disturbance in the environment of pond. To combat this, different antibiotics and chemicals are being used which are reported to be not environment friendly. Of late, a new and unique biotechnological product called "Probiotics" is being used widely by all the shrimp farmers worldwide, which is found to be more effective and environmentally safe also. In the present study 2 probiotics were used in a small 0.7 ha shrimp farm near Pattukottai in Tamil Nadu State for one culture period for the management of pond environment and also the gut ecology of *Penaeus monodon*. The environmental parameters analysed were within the acceptable limits. It was evident from the results that the production was better in the experimental pond where the probiotics were used. The biological parameters such as the average body weight, FCR and total harvest achieved were better in the experimental pond than the control pond, all due to congenial environment, which obtained in the former mainly due to the use of probiotics.

Key words : Probiotics, Environment, Shrimp farming.

Introduction

Bacterial and viral diseases have devastated shrimp aquaculture in Asia in last few years. Indiscriminate use of antibiotics and antimicrobial chemicals has worsened the situation instead of creating conducive disease free culture environment. Of late research is oriented towards the use of environmentally safe products to combat the disease, which not only enhance the immune system of shrimps but also keep the environment clean and stress free. The new biotechnological product, which is called as 'Probiotics' and means for life, contains beneficial microbes that are naturally occurring in water, sediment and shrimp or fish guts. When added (at high population densities) in the culture ponds or in the feed, they displace pathogenic (disease causing) bacteria by competitive exclusion and keep the environment clean. In the present study the role of commercial probiotics on the environment of shrimp ponds was studied.

Materials and Methods

The present experiment was conducted in a private shrimp farm situated near Pattukottai on

the southeast coast of Tamil Nadu state. This farm has seven ponds with a total water spread area of 4.0 ha. For the present study two ponds (experimental and control) each of 0.7 ha water spread area were selected. Shrimp seeds (*Penaeus monodon*) were purchased from commercial hatchery near Marakanam after screening them by PCR technique for White Spot Syndrome Virus (WSSV) in a private testing laboratory at Chennai. The two ponds were stocked at a density of 8.6 shrimps / m². Seawater was pumped into the ponds and usual culture practice was followed. The shrimps were fed with "Luxe Water Base" commercial feed throughout the culture period.

In the present study, two brands of probiotics were used namely "Environ AC" (Wockhardt) for pond water treatment and "Bio tech" (C P) for gut microbial population enhancement. The beneficial bacteria present in the pond water treatment consist mainly of non-pathogenic microorganisms such as *Bacillus subtilis*, *B. licheniformis*, *Pseudomonas aeruginosa*, *P. putida*, *P. denitrificans*, *Alcaligenes* sp., *Lactobacillus lactis*, *L. helveticus*, *Nitrosomonas* sp., and live yeast *Saccharomyces*

cerevisiae. The gut microflora probiotics consist of sporulating *Lactobacillus* at a strength of 6 billion viable spores per gram.

Twenty Kg of "Environ AC", the environmental probiotics were applied in the 0.7 ha experimental pond initially when the water depth was 50 cm. Then the water level was raised to 1 meter and after that 5 Kg of probiotics were used once in 10 days till the harvest. Minimum of 5 – 10 % water exchange was done prior to each application of probiotics. No probiotics were used in the control pond, 5 to 10% water exchange was done once in 10 days upto 60 days, and then 72% exchange was done once in a week until the harvest.

Similarly, the oral probiotics "Bio-tech", were given regularly to the shrimps by mixing this product using fish oil at the level of 2 g per kg of feed. This feed was given for three days in the evening (6 p.m.) with a gap of 10 days interval from 15 days of culture (DOC) till harvest.

Water quality parameters such as salinity, temperature, pH, dissolved oxygen and

transparency were measured using "YSI 85" DO meter and Secchi disc. Ammonia, nitrite and nitrate were measured using "HACH" analysis kit. Microbial analyses were carried out with samples collected using sterile glass tubes and analysed in a private laboratory at Pattukottai.

Shrimps from both the ponds were sampled regularly once in 10 days for estimating the growth rate, health condition etc. by cast netting. Similarly water samples were also taken for plankton analysis.

Results

During the period of culture, the environmental parameters as salinity (29-34ppt), temperature (28- 30°C), pH (8.1-8.5), dissolved oxygen (3.15- 6.80 ppm) and transparency (45-60 cm) were within the acceptable levels, in the shrimp ponds.

Table 1 shows the harvest details of *Penaeus monodon* culture in the control and experimental ponds. The better culture performance in the experimental pond is quite

Table – 1 : Harvest details of *Penaeus monodon* from the control and experimental ponds.

Details	Control pond	Experimental pond
Total water spread area	0.70 ha	0.70 ha
Total seeds stocked	60,000	60,000
Stocking density	8.6/m ²	8.6/m ²
Total yield	1,062 kg	1,775 kg
ABW	27.8 gm	34.0 gm
Survival	63.70 %	87.00 %
Days of culture	140 days	138 days
Total feed used	1,773kg	2,450 kg
FCR	1.67	1.38

evident. The application of probiotics proved its worthiness through way of better pond environment (Table 2). The yellow and green colonies, which were noticed in the experimental pond initially, were eliminated after the use of probiotics while such colonies increased in the control pond. Further, the ammonia level was below the detectable level in the experimental pond.

During the culture period the plankton bloom was very well maintained in the

experimental pond, whereas in the control pond, frequent crash was noticed followed by pond bottom deterioration, which led to poor animal health and comparatively less survival in the control pond. Besides that, growth of lab-lab was noticed in the control pond, which caused lot of problems, as they tend to float and get accumulated near the corners. But this kind of algal growth and other problems were not encountered in the experimental pond. In the experimental pond, shrimps were found to be healthy all the time and also they were clean

without any fouling or necrosis. Therefore there was higher survival here.

Qualitative analysis of pond water revealed rich diatom and zooplankton population with diversified species throughout the culture period in the experimental pond whereas very few species of diatom as *Nitzschia* sp., *Navicula* sp., *Prorocentrum micans* and *Peridinium* sp.

dominated in the control pond. The population of dinoflagellates was found to be dominant during most of the time and zooplankton species were poor in the control pond. However, in both the ponds, no viral disease symptom was noticed during the culture period except the external fouling and necrosis in the walking legs and chronic soft/loose shell found in the control pond.

Table - 2 : Environment of the control and experimental ponds

Parameters	Control pond		Experimental pond	
	Initial	At harvest	Initial	At harvest
Total viable count (CFU/ml)	4.7×10^3	3.7×10^4	3.8×10^3	1.7×10^2
Yellow colony count (CFU/ml)	1.2×10^1	2.3×10^2	0.7×10^1	ND
Green colony count (CFU/ml)	0.3×10^1	1.7×10^1	0.8×10^1	ND
Ammonia (ppm)	ND	0.07	ND	ND
Nitrite (ppm)	0.12	0.18	0.10	0.05
Nitrate (ppm)	0.64	0.22	0.18	ND

ND - Not Detectable

Discussion

The outbreak of disease in the shrimp farm has become one of the biggest problems confronting the growth of shrimp farming sector. The environmental concerns arising out of shrimp farming practices should also be addressed to as the very existence and sustenance of this important economic activity hinge on the soundness of the environment. Use of probiotics is reported to be advantageous on both these counts.

Application of probiotics was found to improve the water quality parameters, also the condition in the pond bottom thereby enabling the successful culture and harvest. Published information on the influence of probiotics on the pond environment and growth of shrimps is scanty. The importance of probiotics as an emerging concept in shellfish nutrition and disease control was highlighted by Mohamed (1995). Mohan and Shankar (1997) observed that the initial viral load in the water and seed followed by secondary infection play a vital role in deciding the rearing success. Ravi *et al.* (1998) ascertained the efficiency of probiotics on the growth of the second most important cultivable shrimp species, *Penaeus indicus*. They have

further stated that by maintaining the water quality parameters at optimum level, probiotics is reported to be helpful in enhancing the growth rate among the cultured organisms. This is quite evident in the present observation (experimental pond) also. The pH value also increased to the required range (8.1- 8.5) as probiotics has the capacity to control acidity. Thus probiotics was found to be useful in maintaining the pond water pH at the desired level.

Ammonia is the main end product of protein catabolism in organisms and is excreted through gills. It is also produced by the decay of organic matter. Under anaerobic conditions, nitrate is also reduced to ammonia. In the present observation, there was no chance for such situation as the pond water was aerated so also probiotics was applied. The decay of organic matter accumulated at the bottom of the pond through the source of uneaten food, and faecal matter were very low and minimal as the density of shrimp population was moderate. In cultures, the ammonia level should be less than 1 ppm. In the present observation, both in the control and in the experimental shrimp ponds, its levels were below this mark (Table 2). However, it could be seen that there was a built-up of ammonia in the

control pond (0.7 ppm) with the progress of the culture operation. But in the experimental pond, ammonia level remained below the detectable limit. This reduction in ammonia is mainly due to the application of probiotics. Similarly, the reduction in the nitrite and nitrate levels (planktonic bloom) was mainly due to the usage of probiotics in the experimental pond.

The maintenance of gastrointestinal tract is *sine qua non* for disease free sustainable culture operation. If a healthy microbial flora of the gut is maintained, it leads to better health, better digestion, better growth and production of shrimps. Any bacterium to survive has to attach itself to a host cell. If the bacteria do not get enough space to attach, it ultimately dies or removed from the gut. *Lactobacillus* is a quick multiplying and sporulating type when exposed to unnatural conditions like high or low temperatures and excess acidity. Conducive bacteria multiply rapidly and attach itself to the entire intestinal wall. There is no space left out for the pathogenic bacteria to attach. They are thus driven out as microbes against microbes and the process is through "Competitive exclusion". The present study has also clearly demonstrated the usefulness of oral probiotics in enhancing quick growth and disease free culture of the tiger shrimp, *Penaeus monodon* in the experimental pond.

In crustaceans, growth is closely associated with moulting. Under optimal

environmental conditions, organisms, in particular penaeid shrimps grow fast and moult fast. This was very much evident during the present observation. As the "Environ AC" probiotics improved the water quality parameters, the shrimps molted significantly faster in the experimental pond than the control pond. The increased production rate and disease free nature of the cultured organisms of *Penaeus monodon* can be attributed to the oral probiotics which interacted nicely in the gut ecology in a passive manner as already demonstrated in the live stock production.

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Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants.

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Abstract : Excess of cadmium (Cd) induced changes in oxidative scenario and water status of plants viz., total water content, specific water content, water saturation deficit (WSD) and transpiration of *Brassica juncea* plants grown in soil pot culture. Although lower and marginal levels of excess cadmium (100 and 250 ppm) improved growth but higher levels (500 ppm) caused significant suppression. Significant accumulation of proline, an indicator of water stress, occurred at higher level of Cd. Gradual increases in activities of certain antioxidant enzymes such as catalase and peroxidase alongwith increased lipid peroxidation are suggestive of disturbed oxidative metabolism. Taking together, the deleterious effects of Cd and its effects on oxidative metabolism clearly indicate enhanced generation of reactive oxygen species (ROS) to be instrumental in producing toxic effects of Cd. The excess levels of Cd also decreased the concentrations of soluble protein and chlorophylls and increased the ratio of chlorophyll a/b.

Key words : Cadmium toxicity, *Brassica juncea*, Water relations, Catalase, Peroxidase, Lipid peroxidation, Proline, Oxidative stress.

Introduction

Cadmium is one of the most toxic heavy metal pollutants for human beings, animals and plants. It enters in the environment mainly from industrial processes and phosphorus fertilizers and then is transferred to the food chain (Wagner, 1993). When accumulated in the plant tissues, it causes alterations in catalytic efficacy of enzymes (Van Assche and Clijsters, 1988; Somashekaraiah *et al.*, 1992; Romero-Puertas *et al.*, 1999. Piqueras *et al.*, 1999), damage to the cellular membranes (Fu and Brouillette, 1987) and inhibit the root growth (Wilkins, 1978). These changes result in inhibition of chlorophyll biosynthesis and photosynthesis (Singh and Singh, 1987), mineral nutrient uptake (Greger and Lindberg, 1987) and water stress (Barcelo and Poschenrieder, 1990 and Kastori *et al.*, 1992). Information on plant water relations and oxidative metabolism, which constitute a fundamental mechanism in plant's life and they are affected due to metal phyto-toxicity, is still scanty. The present investigation relates the effects of excess Cd on plant water relations and oxidative metabolism in mustard plants grown in soil pot culture under glasshouse conditions.

Materials and Methods

Plant material and culture condition : Indian mustard (*Brassica juncea* L. cv. Varuna) plants were grown in soil pot culture in a glasshouse. The soil was air dried and mixed thoroughly. Nearly 2.5 kg of soil was filled in bitumen painted clay pots. The physico-chemical characteristics of soil are given in Table I. There were six replications for each treatment. Seeds were sown in polythene trays and watered with deionised water. The seedlings were transplanted in clay-pots filled with soil on 10th day after sowing. A basal treatment of 200 mg kg⁻¹ soil as Ca(NO₃)₂ and 100 mg P kg⁻¹ soil as KH₂PO₄ and 100 mg kg⁻¹ soil as MgSO₄ was applied to all plants. The pots were randomized every 4 to 5 days and watered daily to field capacity. After 30 days of growth, pots were grouped in 4 lots each with 6 pots. The first lot was treated as control and no cadmium was supplied. Other 3 lots were treated with cadmium @ 100, 250 and 500 ppm as CdCl₂. The glasshouse conditions during experiment {light intensity (PAR), temperature (maximum and minimum) and humidity ranged from 960 to 1400 $\mu\text{E m}^{-2} \text{s}^{-1}$, 24 to 28 and 10 to 18°C and 40 to

50% (9.00 AM) respectively}. The average day length during the period was 10.5 ± 0.2 hours.

Growth, diffusive resistance (DR), transpiration (E) and water status : Data were recorded as plant height and fresh and dry matter yields (oven dried at 70°C for 24 hours). At 4 and 8 days of Cd treatment, measurements of DR and E were made under glasshouse conditions between 9 to 10 AM on the abaxial surface of fourth expanded leaf using a Li-Cor (Lincoln, NE) steady state porometer (Model LI-1600). The same leaves were sampled for measuring water status of the leaf. Determination of water saturation deficit (WSD) was made by measuring fresh and hydrated (incubated in GDW for 3 hours at 10°C in the dark) and oven-dried weights of 30 discs of leaf (3 replicates of 10 discs each). The area of entire leaves was measured with Li-Cor portable area meter (Model LI-3000 A).

Chlorophylls, proline and soluble proteins : Chlorophyll content was measured according to method of Arnon (1949). The concentration of free proline was determined in fresh leaf tissue with acid ninhydrin complex in toluene (Bates *et al.* 1973). Soluble protein was estimated with a colorimetric method using folin phenol reagent (Lowry *et al.* 1951). Bovine serum albumin (BSA) served as a standard.

Enzyme assays and lipid peroxidation : Ten percent homogenate of fresh finely chopped, and pre-chilled leaf lamina was prepared in phosphate buffer. The homogenate was then centrifuged and supernatant was used for enzyme assays. Catalase activity was measured as described by Bisht (1972) and peroxidase activity by modified method of Luck (1963). The activities of enzymes were expressed per milligram of soluble protein in the enzyme extracts. Lipid peroxidation in the leaf tissue was determined in terms of malondialdehyde (MDA, a product of lipid peroxidation) content by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). The leaf tissue was homogenized in 0.1% (w/v) trichloroacetic acid (TCA) following centrifugation at 10,000 g for 5 minutes. Supernatants were treated with 0.5% (w/v) TBA [prepared in 20% (w/v) TCA] at 95°C and cooled

quickly. MDA concentration was determined after subtracting optical density for nonspecific absorbance (600 nm) from the absorbance values at 532 nm, using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

The data have been analysed for its statistical significance at $P = 0.05$ by analysis of variance.

Results and Discussion

Plant growth and visible effects : Plants receiving lower and marginal level (100 and 250 ppm) Cd exhibited non-significant increase in fresh and dry matter yields but at higher level (500 ppm) of Cd, significant reduction in plant height (Fig. 1) and fresh and dry matter was observed (Table 2). Initiation of wilting was observed in plants receiving 500 ppm of Cd at 4th day of treatment. At this level white necrotic patches appeared that increased in area as the duration advanced. The chlorosis started from the margins of younger leaves and subsequently progressed toward midrib of leaves in both marginal and higher levels (250 and 500 ppm) of Cd. The marginal growth of leaf lamina in these treatments was restricted resulting in downward curling of leaves.

Water relations : Leaves of mustard plants receiving lower and marginal levels (100 and 250 ppm) of Cd exhibited non-significant increase in total water content, specific water content and lower WSD. These parameters decreased significantly in leaves of plants receiving 500 ppm of Cd (Table 2). The lower and marginal levels of Cd (100 and 250 ppm) decreased the DR and concomitantly increased E while at higher level (500 ppm) of Cd increased DR and decreased E (Table 3).

Chlorophylls, proline and soluble proteins : The chlorophyll concentration decreased with increasing Cd and the chlorophyll a/b ratio increased significantly with increasing Cd (Table 4). The proline content registered an increase with increasing level of Cd (Table 2). Concentration of soluble proteins decreased with increase in Cd supply (Table 4).

Activity of antioxidant enzymes and lipid peroxidation : The activity of antioxidant

enzymes viz. catalase and peroxidase increased significantly with increase in Cd supply (Table 4).

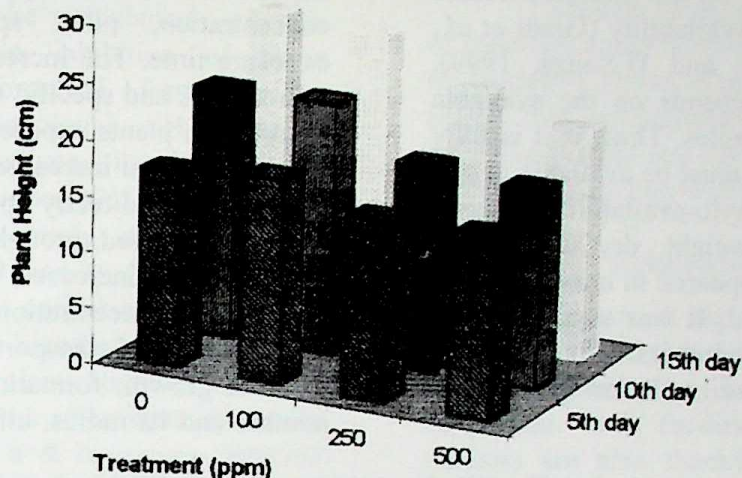


Fig. 1 : Effect of cadmium on plant height of mustard (*B. juncea*) plants growth in soil pot culture at 5, 10 and 15 days after cadmium treatment.

Table – 1 : Physico-chemical characteristics of soil used as culture medium for the pot culture experiment.

Physical character		Chemical character	
Bulk density (gm lit ⁻¹)	1.44	pH	8.36
Particle density (gm lit ⁻¹)	2.00	Electrical conductivity (d Sm ⁻¹)	0.16
Porosity (%)	28.00	Calcium carbonate (%)	0.5
Saturation percentage	37.50	Organic matter (gm kg ⁻¹)	6.86
Water holding capacity (%)	24.50	Nitrogen (%)	0.05
Texture sand (%)	32.00	Potassium (ppm)	120.0
Silt (%)	57.00	Copper (ppm)	2.55
Clay (%)	11.00	Zinc (ppm)	3.15
Textural class	Silt loam	Iron (ppm)	10.46
		Manganese (ppm)	5.54

Table – 2 : Effect of Cd on fresh and dry weight, water contents [total water content (TWC), specific water content (SWC), water saturation deficit (WSD)] and proline concentration in leaves of mustard (*B. juncea*) plants grown in soil pot culture.

Treatment (ppm Cd)	Fresh weight (g plant ⁻¹)	Dry weight (g plant ⁻¹)	Water contents			Proline (μmol g ⁻¹ fresh wt.)
			TWC (g leaf ⁻¹)	SWC (g dm ⁻²)	WSD (%)	
Control	5.13	0.645	1.54	1.79	6.5	0.342
100	6.39	0.725	2.01	2.10	5.5	0.340
250	5.51	0.648	1.88	1.94	6.0	0.352
500	2.84	0.336	0.57	0.79	15.7	3.548
LSD _{0.05}	0.59	0.45	0.36	0.56	0.88	0.660

The MDA content (an index of lipid peroxidation) also increased with increasing supply of Cd (Table 3). These observations clearly suggest an enhanced generation of free radicals and thereby causing oxidative stress.

The insignificant increase in fresh and dry weight of plants receiving lower and marginal level of Cd (100 and 250 ppm) supply may possibly, be attributed to meager availability of Cd in the soil relatively at higher pH (8.36). The

soil pH is a primary factor influencing the availability of Cd and therefore increased sorption of Cd with increasing pH on soil particle surfaces would reduce Cd phyto-availability (Grant *et al.*, 1998 and Rarnchandran and D'Souza, 1999). Sorption of ions also depends on the available surface area on clay micelles. Thus, if it is fully saturated, the metal ions must be available in soil solution with resultant phyto-availability of metal ions. Decreased fresh weight, dry weight and visual toxic symptoms appeared in mustard plants receiving 500 ppm of Cd. It was suggested that uptake of Cd by plants increases with increasing concentration of Cd in soil solution (Mullins *et al.*, 1986).

The effects of heavy metals on plant water relations are quite complex and are dependent on the type of metal and its concentration, plant species, genotype and exposure time. The increased DR and WSD and decreased E and specific water content have been observed in plants exposed to higher level of Cd. The heavy metal increased the stomatal resistance not only when directly applied on guard cells but also when applied through the roots (Lanoreux *et al.*, 1978). The increased WSD, decreased suggest that high Cd concentration probably decreased the water uptake and transport as heavy metals inhibit the root growth, formation of root hairs, vessel number and its radius, all of which together lead

Table - 3 : Effect of Cd on transpiration (E), diffusive resistance (DR) and lipid peroxidation in the leaf tissue of mustard (*B. juncea*) plants grown in soil pot culture.

Treatment (ppm Cd)	Transpiration ($\mu\text{g cm}^{-2} \text{sec}^{-1}$)	Diffusive resistance (s cm^{-1})	Transpiration ($\mu\text{g cm}^{-2} \text{sec}^{-1}$)	Diffusive resistance (s cm^{-1})	Lipid peroxidation ($\mu\text{mol MDA g}^{-1} \text{fresh wt.}$)
	4th day		8th day		
Control	3.50	3.43	2.91	2.99	4.68
100	4.84	2.25	5.39	2.15	5.05
250	3.60	3.55	4.75	1.93	6.12
500	0.38	72.50	0.40	71.43	8.68
LSD _{0.05}	0.48	0.62	0.57	0.63	1.36

Table - 4 : Effect of cadmium on concentrations of chlorophylls, soluble proteins and activities of catalase and peroxidase of mustard (*B. juncea*) plants grown in soil pot culture.

Treatment (ppm Cd)	Total chlorophyll ($\text{mg g}^{-1} \text{fresh wt.}$)	Chlorophyll a/b ratio	Soluble protein ($\text{mg 100 mg}^{-1} \text{fresh wt.}$)	Catalase (Diffusive resistance ($\mu\text{mol H}_2\text{O}_2$ decomposed $\text{mg}^{-1} \text{protein}$))	Peroxidase (unit* $\mu\text{g}^{-1} \text{protein}$)
Control	2.742	2.838	4.26	479	9.25
100	2.369	3.091	3.92	571	24.38
250	1.958	3.211	3.88	567	27.73
500	1.251	3.328	3.60	608	32.19
LSD _{0.05}	0.42	0.38	0.83	8.71	2.39

*Change of optical density of 0.01 in the sample reaction mixture over the blanks per minute of reaction time.

to increased resistance to the water flow into and within roots (Barcelo and Poschenrieder, 1990). The slight or insignificant increase in the rate of E and specific water content, decrease in DR and WSD at low Cd supply levels are similar to the results of Hagemeyer *et al.* (1986). Low Cd concentration may decrease root growth without

producing any toxic effect in the leaves. However, in leaf Cd can influence water relation by causing changes in photo-assimilate partitioning or increase the photosynthesis, which bring about increased turgor of leaves, stomatal opening and E (Barcelo and Poschenrieder, 1990).

The concentration of chlorophyll a and b decreased significantly in plants subjected to high level of Cd treatment. The decrease in chlorophyll concentration might be attributed to the involvement of Cd in inhibition of heme biosynthesis and chlorophyll formation by interacting with the functional -SH group of sulphhydryl requiring enzymes involved in the pathway (Nandi and Shemin, 1968 and Stobart *et al.*, 1985). Somashekaraiah *et al.* (1992) suggested that increased activity of lipoxygenase in response to Cd might contribute to decreased level of chlorophylls. Significant increase in chlorophyll a/b ratio in plants supplied Cd could be explained on the basis of relative decreases noticed for chlorophyll a & b. It was observed (data not given) that magnitude of decrease in chlorophyll b was greater than chlorophyll a. This relative difference is reflected in the increased ratio. The greater decrease in chlorophyll b due to excess Cd may have resulted either from any or in combination of these mechanism (a) greater degradation of chlorophyll b, (b) its enhanced conversion to chlorophyll a or (c) reduced conversion of chlorophyll a to chlorophyll b. The occurrence of such interconversions has been reported by Ito *et al.* (1996).

The increased accumulation of proline in the leaf tissue might be caused by increase in WSD in plants exposed to higher doses of Cd. Proline is accumulated by several plants under various stress conditions such as low temperature (Stefl *et al.*, 1978) nutrient deficiencies (Sharma and Sharma, 1987 and Sharma *et al.*, 1995), toxicity of heavy metals (Kastori *et al.*, 1992 and Bassi and Sharma, 1993) and water stress (Carceller and Fraschina, 1980). The increased activity of catalase and peroxidase in plants exposed to higher level of Cd is in accordance to the results of Romero-Puertas *et al.* (1999). The increased activity of catalase and peroxidase, the prime antioxidant enzymes responsible for the detoxification of H_2O_2 was conducive to improved tolerance to Cd toxicity. Cd causes peroxidation of essential membrane lipids probably by generation of ROS. In fact, increased lipid peroxidation in plants treated with Cd was noticed and is in accordance of earlier observation

(Somashekaraiah *et al.*, 1992 and Piqueras *et al.*, 1999).

Exposure of mustard plants to high levels of cadmium could be considered as abiotic elicitor of oxidative stress even in soils with high pH (8.36) that subsequently damage the essential membrane lipids and also affects uptake of water and thereby causing water stress in plants.

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Insecticide susceptibility status of *Aedes aegypti* to DDT and dieldrin in desert and non-desert parts of Rajasthan.

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Abstract : Seasonal prevalence and insecticide susceptibility tests were carried out on the adults of *Aedes aegypti*, the vector of dengue and dengue hemorrhagic fever (DF/DHF), in three desert (Bikaner, Jaisalmer and Jodhpur) and three non-desert (Alwar, Ajmer and Jaipur) districts of Rajasthan. Among the three species viz. *Ae. aegypti*, *Ae. vittatus* and *Ae. w-albus* encountered during the survey the former was the most prevalent species. Studies were carried out both in rural and urban areas against different concentrations of two organochlorines (DDT and Dieldrin). In rural areas resistance has been observed with DDT and dieldrin both in desert and non-desert parts while an intermediate resistance has been observed in the urban areas with both the insecticides which required further verification. Resistance was more pronounced in rural areas as compared to urban areas. LC_{50} along with regression equation and Chi-square values have been determined for both the insecticides.

Key words : Insecticides, Susceptibility, *Ae. aegypti*, Desert, Non-desert.

Introduction

Several outbreaks of febrile illness by dengue viruses (DEN) have been reported from different parts of Rajasthan state (Padbidri *et al.*, 1973; Ghosh *et al.*, 1974; Chauhan *et al.*, 1990), due to widespread prevalence of the vector not only in urban areas but also in rural areas (Verma *et al.*, 1991; Bansal *et al.*, 1994). Rapid and unplanned growth of residential areas in the peripheral surroundings, wet cultivation with sewage water, large water bodies in low lying areas contaminated with sewerage and animal waste all combine and aggravate the breeding of mosquito vectors, especially the culicines. Scarcity of drinking water particularly in the desert region of Rajasthan state often necessitates water storage practices in cemented tanks, clay pitchers and shallow wells, which is directly related to the potential breeding of *Ae. aegypti*, a culicine vector responsible for the transmission of dengue and dengue hemorrhagic fever.

Although some studies on the insecticide susceptibility of this mosquito have been done in different parts of India but very less work has been done in the desert and non-desert parts of

Rajasthan. Therefore, studies were conducted in order to know the status of DDT and dieldrin resistance in this species.

Materials and Methods

Rajasthan state due to its geographical location has naturally been divided into desert and non-desert parts. Both these regions have their own climatic conditions, which have a direct bearing on the prevalence and seasonal distribution of different disease vectors. In order to carry out the present studies, three desert (Bikaner, Jaisalmer and Jodhpur) and three non-desert districts (Alwar, Ajmer and Jaipur) were selected and surveys carried out both in rural and urban areas. Collection was made from inside the human dwellings, cattle sheds, clay pitchers, tyre dumps, tree holes and shallow wells. Adults were collected with the help of an aspirator supplied by W.H.O. and kept in Barraud cages provided with cotton pads soaked in 10% glucose solution.

Susceptibility tests were carried out on the engorged females (temp. $28 \pm 2^\circ\text{C}$ and RH 70-80%) as per prescribed procedure (WHO, 1970). DDT (0.5 to 4.0%) and dieldrin (0.05 to 0.4%)

impregnated papers supplied by WHO NAMP (Delhi) were used. In each test, 20-25 females of *Ae. aegypti* were exposed to above insecticide papers for a period of one hour during which no feed was given. Three to five replicates were used and percent mortalities were recorded 24hr after exposure. Whenever, control mortality exceeded 5%, the corrected mortality was calculated by Abbott's formula. LC50 values, were calculated from the log concentrations probit regression lines. Susceptibility status was determined as per WHO criteria (WHO, 1986) as follows: Insecticide x maximum exposure period, viz. DDT (4.0% x 1hr) and dieldrin (0.4% x 1hr). Susceptibility status was determined by percent mortality viz. mortality >98% = Susceptible (S); 80-98% = Intermediate resistance or verification required (V) and <80% = Resistant (R).

Results and Discussion

Results of the survey and insecticide susceptibility tests carried out in desert and non-desert parts have been given in Fig. 1 and Table 1. From the plot, it is clear that *Ae. aegypti* was present throughout the year, except low density during peak summer and winter months, with two population peaks, first during March-May and 2nd during Sept.-Oct. with a density as high as 38 PMH. However, the other two species *Ae. vittatus* and *Ae. w-albus* were present only during the monsoon and post monsoon period. High density and prevalence of *Ae. aegypti* which is a known vector of DF/DHF throughout the year clearly suggest that how this disease maintains itself in the endemic form or its sudden eruptions in the epidemic forms in Rajasthan. An extreme of temperature, during both summer and winter

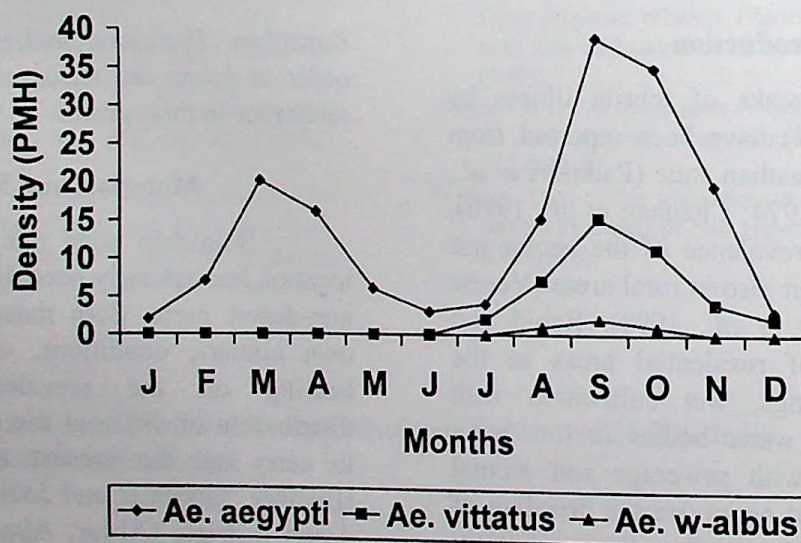


Fig. 1 : Monthly prevalence of *Aedes* spp. in urban areas of Bikaner district.

seasons, however, maintains the vector population under natural control. Prevalence and seasonal distribution of this species in rural and urban areas of both desert and non-desert parts clearly suggest that how variations in temperature, relative humidity, geographical location of a place, its fauna and flora and insecticide spray history has an influence on the vector species.

Susceptibility tests were conducted only on *Ae. aegypti* because of its high density and the primary vector of DF/DHF and the results are tabulated in Table 1. Percent mortality was found to increase with the increase in concentration of both the organochlorines. On exposure to different concentrations of DDT (0.5 to 4.0%) and dieldrin (0.05 to 0.4%), a resistance has been observed with both the insecticides in the rural

areas while an intermediate resistance was observed in the urban areas at the diagnostic dose and time both in desert and non-desert parts. Resistance was more pronounced in rural areas with both the compounds as compared to urban areas. LC_{50} values for DDT and dieldrin were almost double in the rural areas than in the urban areas (table 1) in both desert and non-desert parts, which may be due to the continuous spray of DDT in the rural areas, which resulted in the gradual development of insecticide resistance. Bansal and Singh (1995) showed inter-mediate resistance in this species with DDT and dieldrin

while working with organochlorines (DDT and dieldrin), organophosphate (malathion and fenitrothion), carbamate (propoxur) and synthetic pyrethroids (permethrin) in district Bikaner, Rajasthan. The first case of DDT resistance in the field population of this species was discovered in Jharia, Bihar, in 1965 (Azeez, 1967). Since then many instances of complete or intermediate resistance to DDT in natural population of *Ae. aegypti* have been reported from different parts of the country (Raghavan *et al.*, 1966; Madhukar and Pillai, 1970; Brown and Pal, 1971; Kaul *et al.*, 1976).

Table - 1 : Susceptibility status of *Aedes aegypti* to DDT and dieldrin in rural and urban areas of desert and non-desert districts.

Insecticide tested	Conc. used	Percent mortality		Susceptibility status	Regression equation	Chi-square (X ²)	LC ₅₀ ± S.E (Fiducial limits)
		(D)	(ND)				
Rural Areas							
DDT	0.5	12.4	10.2	(R)	1.70X+0.83	0.60	2.8520±0.0123 (D) (1.8880-4.3070)
	1.0	20.0	21.4		1.31X+1.57	0.92	4.1860±0.0137 (ND) (2.2530-7.7760)
	2.0	37.1	39.6				
	4.0	64.3	45.4				
Dieldrin	0.05	20.4	18.4	(R)	1.52X+3.07	0.14	0.1837±0.0124 (D) (0.1205-0.2800)
	0.2	50.1	36.2		1.22X+3.18	0.73	0.3114±0.0136 (ND) (0.1711-0.5666)
	0.4	72.2	60.0				
Urban Areas							
DDT	0.5	22.8	15.7	(V)	1.81X+1.23	0.27	1.2050±0.0117 (D) (0.8841-1.6430)
	1.0	48.2	30.0		1.86X+0.78	0.61	1.8400±0.0118 (ND) (1.3230-2.5570)
	2.0	64.4	48.6				
	4.0	82.0	80.0				
Dieldrin	0.05	32.0	24.2	(V)	1.23X+3.68	1.60	0.1185±0.0137 (D) (0.0643-0.2185)
	0.2	56.6	50.0		1.49X+3.21	1.49	0.1581±0.0127 (ND) (0.0998-0.2503)
	0.4	89.4	82.5				

(S) = Susceptible (V) = Verification required (R) = Resistant

(D) = Desert (ND) = Non-desert

LC_{50} = Concentration required for 50% mortality.

Recently Mourya *et al.* (1993) while working on the status of resistance of different populations of *Ae. aegypti* from several villages of Vidarbha and Marathwada region of Maharashtra state also found that both adult and larval stages showed resistance to DDT except the sensitive colony strain. However, no resistance to deltamethrin and

malathion was detected at any stage. Bansal and Singh (1995) also reported 100% susceptibility to malathion and permethrin in the adults of *Ae. aegypti* in Bikaner region of Rajasthan state. High resistance to DDT may perhaps be attributed to the extensive use of this insecticide for malaria control in the country. Grant and Matsumura

(1989) showed that increased DDT resistance in *Ae. aegypti* might be due to the increase in the kinetics of glutathione-s-transferase. The incipient resistance to dieldrin may also be due to the development of cross-resistance to other agricultural pesticides. Some more studies on the biochemical mechanisms of insecticides resistance are needed for confirming the variable responses shown by different species in different areas. Results of the susceptibility tests obtained in the present investigation may very well serve as a baseline data in the formulation of control strategy for this vector in this region.

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Growth and cadmium uptake in barley under cadmium stress.

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Abstract : Effect of various additions of cadmium (5 to 3125 $\mu\text{g g}^{-1}$ air dried soil) was studied on growth and Cd uptake in barley grown in pots containing two soil types- a loamy sand and a sandy loam, during different stages of growth. While lower doses (5 to 25 $\mu\text{g g}^{-1}$) led to an increase, the higher doses resulted in a substantial decrease in barley growth. The plant Cd content increased with an increase in soil applied Cd. A decrease in translocation ratio prevailed at higher soil applied Cd, indicating the existence of an exclusion mechanism.

Key words : Cadmium, Growth, Translocation ratio, Threshold concentration, Critical tissue concentration, DTPA.

Introduction

Cadmium occurs naturally at elevated levels in terrestrial environment in areas with Cd-rich carboniferous shales, and in the vicinity of veins of Pb/Zn mineralization. The soils overlying lead- zinc deposits at Zawar Mines, India have been found to contain Cd in the range of 0.55 to 95.20 $\mu\text{g g}^{-1}$ (Aery and Tiagi, 1985 a and b; 1986). Local Cd enrichment may result from metalliferous mining and smelting, especially of zinc (Aery and Tiagi, 1986), from sewage sludge application (Canet *et al.*, 1998) and the use of contaminated organic and phosphatic fertilizers. Plants take up Cd easily and have been shown to accumulate it (Aery and Tiagi, 1985 b, 1986; Tichy *et al.*, 1997).

The present study investigated the effect of low and high Cd doses in barley, an important Rabi cereal, in two soil types. The main aims of the study were to ascertain Cd- tolerance in terms of growth and grain yield, and to study the Cd-translocation and accumulation and to compare the response of two soil types. The study is also pertinent keeping in view the presence of Zn-Pb-Cd deposits and smelting plants in the vicinity of Udaipur, Rajasthan.

Materials and Methods

Pot culture studies were carried out with six Cd concentrations, replicated thrice and two soil types, a sandy loam, 'A' and loamy sand 'B' (Table 1). Plants were grown in polythene lined

earthen pots of 30 cm diameter containing air-dried soil of each type, with different amounts of $\text{Cd Cl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$, to give concentrations of 0, 5, 25, 125, 625 and 3125 $\mu\text{g g}^{-1}$ of air-dry soil. The experiments were conducted in open air during mid-December to mid-April.

Fifteen seeds were sown equidistantly at 2-cm depth in each pot, and watered on alternate days. After emergence, the plants were thinned to 10 seedlings in each pot. Plant samples were taken at two growth stages i.e. vegetative (before the initiation of flowering) and fruiting (after grain ripening). Observations were recorded on root and shoot length, leaf area, and plant dry weight and grain yield. The plant material was dried in an oven at 80°C for 48 hours. The dried plant material was wet digested with nitric and ternary acid mixture (Jackson, 1967), and the digest was directly atomized in an atomic absorption spectrophotometer (Carl Zeiss, AAS1). After harvesting, soil samples from each treatment were collected and analyzed for DTPA exchangeable Cd.

Results and Discussion

In spite of the non-essentiality of cadmium and its known toxic effects (Aery and Sarkar, 1991), lower concentrations of Cd resulted in an increase in barley growth in both the soil types. While the increase was evident up to 25 $\mu\text{g g}^{-1}$ Cd in soil type 'A', in soil type 'B' it was evident up to 125 $\mu\text{g g}^{-1}$ soil applied Cd. The

increase in root elongation was relatively greater than shoot elongation (Fig. 1). The effect of lower

concentrations on root length was greatest during the vegetative stage, while on shoot length the

Table – 1: Physico-chemical characteristics of the experimental soils.

Parameter	Soil 'A'	Soil 'B'
Water holding capacity (%)	28.255	40.185
Specific gravity	2.39	2.62
Bulk density	1.32	1.26
Porosity (%)	44.68	51.56
Texture	Sandy Loam	Loamy Sand
pH	8.5	8.6
Electrical conductivity (m mho)	0.14	0.32
Organic carbon (%)	0.525	0.675
P ₂ O ₅ (Kg/ hectare)	4.48	39.20
K ₂ O (Kg/ hectare)	131.04	191.88
Extractable Cd ($\mu\text{g g}^{-1}$)	4.2	6.5

maximum effect was during the grain filling stage (Fig 1). No explanation could be given regarding this increase at lower concentrations of Cd. Root growth continued until about grain filling stage

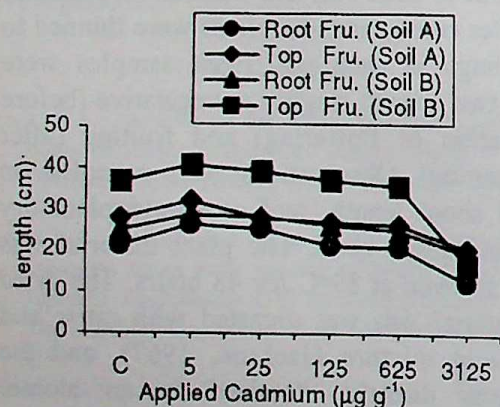


Fig. 1 : Showing the effect of different Cd concentrations on the root-top length and dry weight of barley grown in soil type 'A' during fruiting stage.

weight decreased. However, the shoot continued to grow so that the shoot- root ratio increased steadily throughout the life span of the plant.

The maximum dry weight accumulation in soil type 'A' was observed at $5\mu\text{g g}^{-1}$ Cd addition, in the shoot at grain filling stage and showed 67% increase over the control. In soil type 'B', the maximum shoot biomass was 30.8% higher than the control at $25\mu\text{g g}^{-1}$ Cd addition. In this soil type, higher Cd levels also resulted in an

increase in the root and shoot biomass, over the control, but the increase was less marked than at $25\mu\text{g g}^{-1}$ Cd addition (Fig. 2). Leaf area was also affected by Cd additions. The maximum increase in the leaf area over the control in soil type 'A' (56%) and 'B' (40.25%) was observed during the vegetative stage at $25\mu\text{g g}^{-1}$ Cd addition (Fig. 3). The maximum increase in seed number per ear (68.86%) and seed weight per 100 seeds (76.11%) over the control in soil type 'A' was observed at $5\mu\text{g g}^{-1}$ Cd addition. In soil type 'B', maximum increase over the control in seed numbers (10.82%) and seed weight was observed at $25\mu\text{g g}^{-1}$ Cd level (Table 2).

Higher levels of Cd resulted in a substantial decrease in plant growth. The decrease was evident beyond $25\mu\text{g g}^{-1}$ cadmium addition in case of soil type 'A', and $125\mu\text{g g}^{-1}$ Cd addition in soil type 'B'. At higher Cd levels the lower leaves became chlorotic at an early stage. The chlorosis started from the tip and margins of leaves, and extended inwards and base of the leaves. Simon (1998) observed no effect of Cd on the fresh weight and dry matter accumulation in sunflower grown in sandy loam brown forest soil treated with 1 to 10 mg kg^{-1} of Cd. Although reduced yield was observed at higher Cd levels, the ear setting and

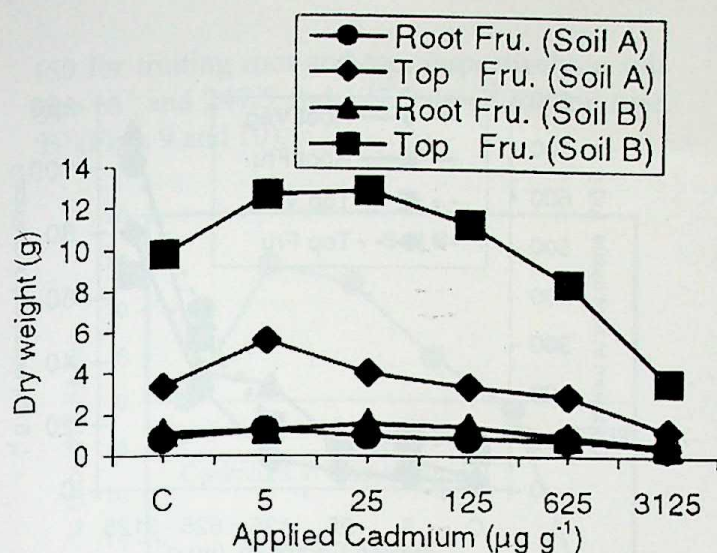


Fig. 2 : Showing the effect of different Cd concentrations on the root-top length and dry weight of barley grown in soil type 'B' during fruiting stage.

ageing were enhanced at higher Cd levels compared to control. Loading rate of cadmium to produce a 10% growth inhibition in terms of dry weight was observed to be 916, 220, 850 and 975 $\mu\text{g g}^{-1}$ for roots and shoots at the vegetative and grain filling stages, respectively in soil type 'A', and 1142, 680, 1171 and 850 $\mu\text{g g}^{-1}$ for soil type 'B' (Fig. 2).

Growth inhibition at higher Cd levels may be linked either to a lower mitotic activity in the root meristematic zone and/or to an inhibition of cell enlargement in the elongation zone as a consequence of decreased cellular turgor (Gabbrielli *et al.*, 1990; Powell *et al.*, 1986) or due to the reduced extensibility of the cell wall (Pandolfini *et al.*, 1992).

Cd uptake : The concentrations of plant Cd increased with an increase in soil applied

Table - 2 : Effect of different cadmium additions ($\mu\text{g g}^{-1}$) on the grain yield (no./ear and wt./100 grains) and percentage increase/ decrease over the control in barley during different stages of plant growth.

Cd level	Soil 'A'			Soil 'B'	
		Seed no.	Seed wt.	Seed no.	Seed wt.
Control	A.M.	3.180	2.010	12.653	3.480
	S.D.	± 0.350	± 0.265	± 0.393	± 0.452
5	A.M.	5.370	3.540	13.730	4.290
	S.D.	± 0.141	± 0.276	± 0.445	± 0.338
	%I/D	68.868	76.119	8.509	23.276
25	A.M.	4.470	2.240	14.030	4.157
	S.D.	± 0.619	± 0.499	± 0.318	± 0.253
	%I/D	40.566	11.443	10.880	19.444
125	A.M.	3.680	1.990	11.960	3.890
	S.D.	± 0.301	± 0.312	± 0.401	± 0.235
	%I/D	15.723	-0.995	-5.479	11.782
625	A.M.	2.440	1.990	8.260	3.120
	S.D.	± 0.296	± 0.366	± 0.260	± 0.346
	%I/D	-23.270	-5.473	-34.721	-10.345
3125	A.M.	1.360	1.590	3.390	3.120
	S.D.	± 0.236	± 0.241	± 0.207	± 0.389
	%I/D	-57.233	-20.896	-73.209	-38.506
r		-0.789	-0.511	-0.941**	-0.984*
r ²		0.62	0.26	0.89	0.82
RE		$y=4.81-0.001x$	$y=2.40-0.00x$	$y=12.72-0.003x$	$y=3.89-0.001x$

Cd (Figs. 3 and 4). In contrast to the observations by Wallace *et al.* (1977), roots accumulated

manifold more Cd than the shoots (Jarvis *et al.*, 1976; Aery and Tiagi, 1986; Simon, 1998). Roots

seem to possess some binding mechanism wherein Cd gets immobile and hence innocuous (Malone *et al.*, 1973). The uptake of Cd increased with age as plants at grain filling stage had more Cd than the vegetative stage plants (Figs. 4 and 5).

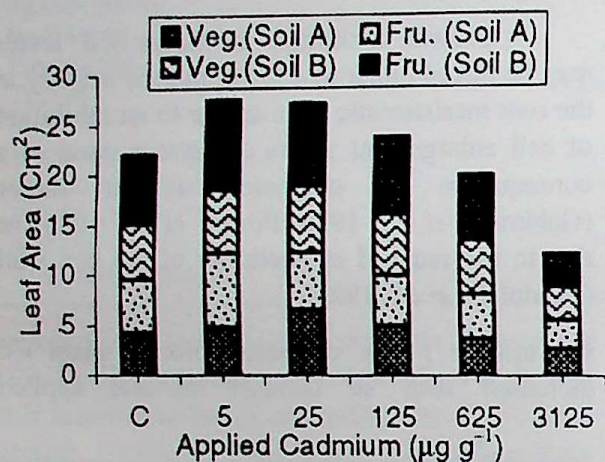


Fig. 3 : Showing the effect of different Cd concentrations on the leaf area of barley grown in soil type 'A' and 'B' during vegetative and fruiting stages of growth.

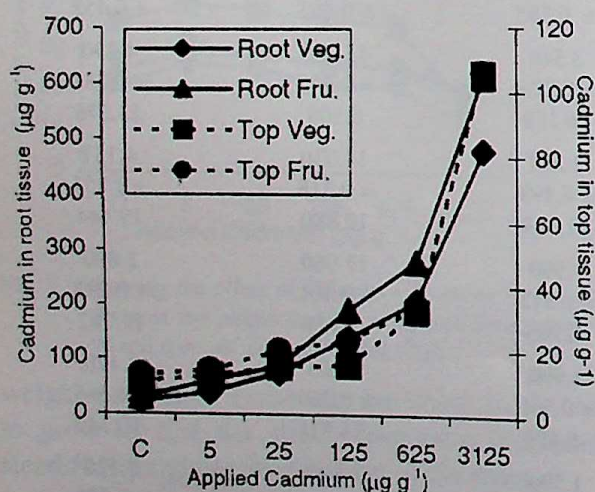


Fig. 4 : Showing Cd contents in barley tissues as a function of applied Cd in soil type 'A'.

Critical tissue concentration : As tissue Cd concentration increased, initially an increase and subsequently a decrease in growth resulted (Figs. 6 and 7). A 10% growth reduction was observed at $50 \mu\text{g g}^{-1}$ in shoot at grain filling stage, in soil type 'A'. In soil type 'B', it was $64.29 \mu\text{g g}^{-1}$.

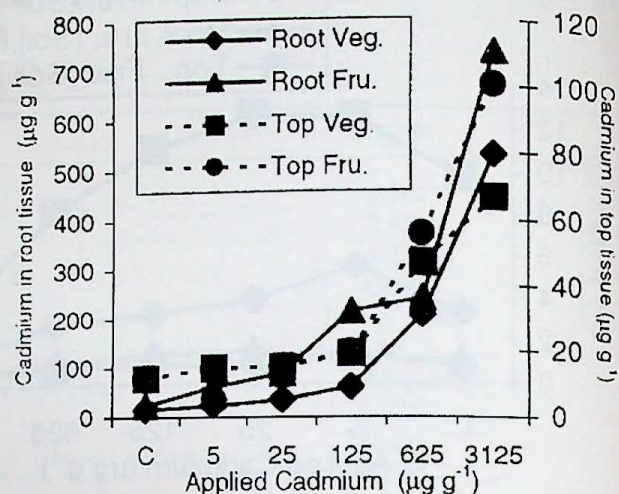


Fig. 5 : Showing Cd contents in barley tissues as a function of applied Cd in soil type 'B'.

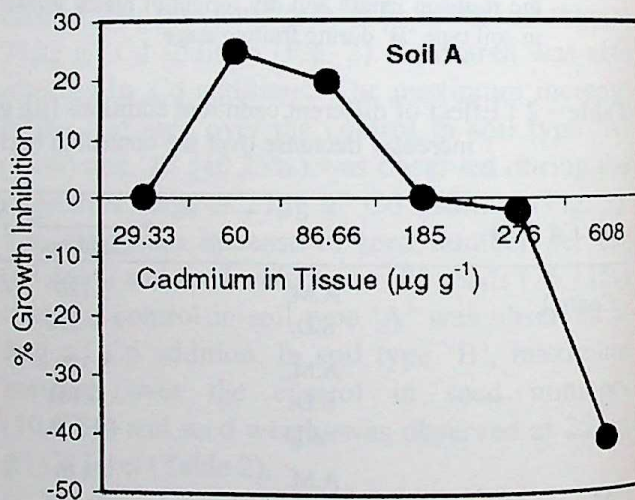


Fig. 6 : Showing percent growth inhibition in barley in soil type 'A' as a function of plant tissue Cd.

Extractable cadmium and threshold concentration : Extractable metal content is a measure associated with availability to plants and uptake (Dang *et al.*, 1990). As the addition of soil applied Cd was enhanced (Fig. 8), a substantial increase in the DTPA exchangeable residual soil Cd occurred (Van Assche and Clijsters, 1988). The amount of DTPA extractable Cd in soil at which growth is reduced by 10% is considered as the threshold concentration of Cd (Dang *et al.*, 1990). It was estimated by plotting DTPA extractable elements as a function of percent inhibition of growth and was found to be 145 and

Growth and cadmium uptake in Barley.

150 for fruiting root and top, respectively in soil type 'A' and 247.5 and 177.5 $\mu\text{g g}^{-1}$ for soil type 'B' (Figs. 9 and 10).

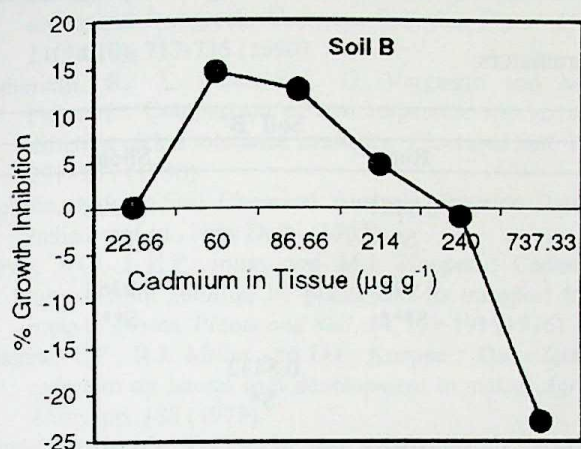


Fig. 7 : Showing percent growth inhibition in barley in soil type 'B' as a function of plant tissue Cd.

Positive significant relationship between plant Cd x residual soil Cd, and soil applied Cd x plant Cd, but a negative and significant relationship between plants Cd x dry weight for both the soil types were observed (Table 3).

For the two soil types studied, uptake of Cd by roots and shoots was always higher in soil type 'A' than in soil type 'B' (Figs. 4 and 5). One of the factors responsible for the lower concentration of Cd in roots and tops of plants grown in soil type 'B' could be due to the higher amount of clay and silt fractions, and organic carbon in soil type 'B' than in soil type 'A' (Table 4), which rendered the metals unavailable to the plant roots. As the plants grown in soil type 'B' showed more vigorous growth than the plants in soil type 'A', the dilution factor (Aery, 2000) due to enhanced growth can be another factor responsible for the lower tissue metal concentration of plants grown in soil type 'B' than that of soil type 'A' (Figs. 1 and 2).

Though the translocation of Cd from roots to tops increased with an increase in substrate metal concentration (Figs. 4 and 5), a decrease in translocation ratio prevailed at higher soil applied Cd concentration (Table 4). Metals are transported from roots to the shoots as organic

metal complexes with citric acid or some other organic acids in xylem and this translocation of metal from

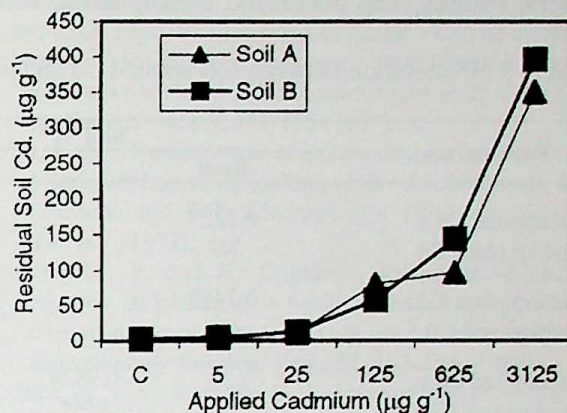


Fig. 8 : Showing the residual soil Cd as a function of applied Cd in two soil types.

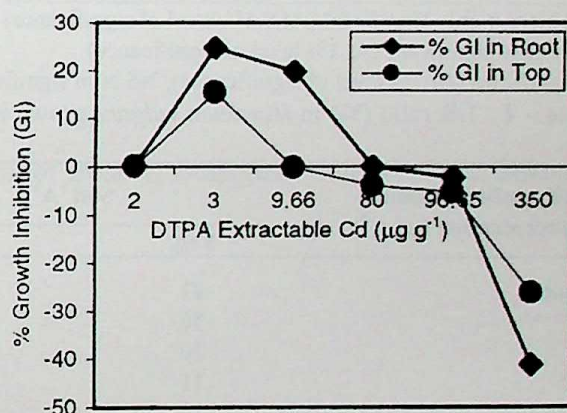


Fig. 9 : Showing the percentage growth inhibition in barley as a function of DTPA extractable Cd in soil type 'A'.

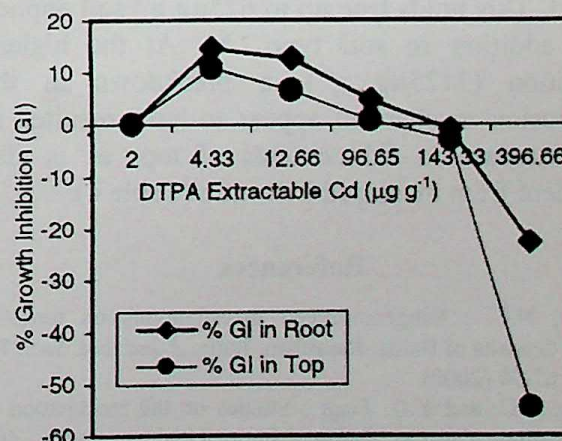


Fig. 10 : Showing the percentage growth inhibition in barley as a function of DTPA extractable Cd in soil type 'B'.

roots to tops is a metabolic process controlled by physico-chemical conditions and application of excessive amounts of metal reduces the translocation ratio (Chino and Baba, 1981). In the present studies, the decreased translocation ratio

at higher Cd additions ($625-3125 \mu\text{g g}^{-1}$) appears to be due to reduced metabolic activities of roots (Tiffin, 1972). Top-root Cd ratio of barely in case of soil type 'A' also indicate that the exclusion mechanism (Baker, 1981) must be responsible for

Table - 3 : Correlation between soil applied Cd and plant growth parameters.

Parameters	Soil 'A'		Soil 'B'	
	Root	Shoot	Root	Shoot
Soil applied Cd × Fruiting plant Cd	0.7257 NS	0.9965 S***	0.8464 S*	0.8308 S*
Fruiting plant Cd × Residual soil Cd	0.7452 NS	0.9885 S***	0.9945 S***	0.9363 S**
Soil applied Cd × Residual soil Cd		0.9824 S***		0.8432 S*
Fruiting plant Cd × Dry weight	-0.818 S*	-0.810 S*	-0.0802 S*	-0.939 S**

S*** Very highly significant (At 0.1% level of significance)

S** Highly significant (At 1% level of significance)

S* Significant (At 5% level of significance); NS Non significant

Table - 4 : T/R ratio (%) in *Hordeum vulgare* grown in soil types 'A' and 'B' at different concentrations of Cd.

Soil applied cadmium Concentration ($\mu\text{g g}^{-1}$)	Soil 'A'		Soil 'B'	
	Veg.	Fruit.	Veg.	Fruit.
Control	43	39	79	51
5	30	22	65	24
25	20	22	43	17
125	11	12	32	9
625	15	12	22	23
3125	17	22	12	14

keeping the Cd content of the tops at a lower level. This holds true up to $625 \mu\text{g g}^{-1}$ soil applied Cd addition in soil type 'A'. At the highest addition ($3125 \mu\text{g g}^{-1}$), a breakdown in the exclusion mechanism appear to have resulted in an increase in Cd contents of tops as is also evident from increased T-R ratio (Table 4).

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Physico-chemical and bacteriological investigation on the River Torsa of North Bengal.

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Abstract : *A few physico-chemical and bacteriological parameters on certain locations of the river Torsa was studied. The major characteristics of Torsa river water were high alkalinity, high concentration of free ammonia with respect to albuminoid ammonia and the presence of bacteria of fecal origin. Marked seasonal variations of the parameters were also observed.*

Key words : *Water analysis, Torsa River, Physico-chemical and bacteriological characteristics, Alkalinity, Free ammonia and albuminoid ammonia, Fecal pollution.*

Introduction

Torsa, an international river, intersects three countries, China (Tibet), Bhutan and India before entering Bangladesh. Torsa, which is also called as 'Amo-chu', is one of the principal rivers in Western Bhutan. The river flows rapidly and follows a confined valley between precipitous mountains. Even in the winter, 'Amo-chu' is a fierce, swift stream. Torsa cuts across in a southeasterly direction and passes by a market town of Phuntsholing on the Indo-Bhutan Border. In the west of Torsa river Baxa-dolomites form striking ridges, this can be seen, from Phuntsholing (Karan and Jenkins, 1967). As it leaves the foothills of Bhutan and enters the undulating Duars plain in the northern part of West Bengal, it widens into a braided channel that also drains the forest cover of Jaldapara wild life sanctuary. Water quality of this turbulent river, therefore, long remained unaltered being determined by its environment, climate, geologic, hydrologic, physiographic, biological and cultural backdrop. The principal water source of wild life, forest tribes and rural communities is often the river Torsa or some combination of well, pond and river water, depending on season and purposes for which water is used.

With the development of roadways connecting Bhutan and India and advent of

modernization of Bhutan in 1950, there have been seen changes in urban and industrial development in and around Phuntsholing lying on the bank of Torsa and adjoining Duars plain in India (Karan and Jenkins, 1967). Indiscriminate dolomite mining in Bhutan hillocks for cement factories leading to large-scale seepage of dolomite in ground and surface water is a well-noted phenomenon (Dutta, 1998; Statesman News Service, 1998). On the other hand, the swelling rural population of this region does not have services of any kind whatsoever, either for potable water or for excreta disposal. The consequent consumption of the river water, which has now degraded, is making the inhabitants of this valley more prone to diseases and health problems. Such observations also put wild life health into question. Furthermore, with industrialization projected to increase many times over the present level, river water pollution will become an even greater concern.

Water quality monitoring of rivers of North Bengal has generally been overlooked for various reasons among which are resources and manpower constraint, institutional inertia, and public apathy due to lack of awareness. In the present investigation, Torsa River was undertaken to examine its water quality for evaluating the impact of various anthropogenic activities.

Materials and Methods

Reagents and other materials : All chemicals were of analytical grade. Deionized water, doubled distilled in glass stills, was used for analytical work. Sterilized sample water bottles were used for collecting water for bacteriological analysis.

Sampling and analysis : Water samples were collected separately for determination of physico-chemical and bacteriological characteristics from three sampling stations (SS-I, Hasimara; SS-II, Falakata; SS-III, Coochbihar) of river Torsa, which are shown in Fig.-1, in January, February, March, May, June, September, October, November and December spanning a period from September 1999 to May 2001. Temperature and pH were measured at the collection sites. The samples were preserved for other parameters in accordance with the Standard methods (APHA, 1989; Manivasakam, 1980). For bacteriological analysis, water samples collected in sterile sample bottles were transported to the laboratory in ice box and minimum elapsed time between collection and analysis in no case did exceed 30 h. Bacteriological analysis consisted of standard plate count, presumptive and confirmatory tests for coliforms and MPN of total coliform, fecal coliform and fecal streptococci (APHA, 1989).

Results and Discussion

Thirty-two samples, consisted of 11, 12 and 9 samples from Hasimara (SSI), Falakata (SSII) and Coochbihar (SSIII) respectively, were collected and studied on monthly basis (except April, July and August) between September 1999 and May 2001. Leaving the peak monsoon months of July and August, the months were grouped as pre-monsoon (March, May and June), post monsoon (September and October) and winter (November to February). The values of various characteristics were shown in Table 1.1 to 1.3.

Air temperature at all spots was recorded regularly throughout the study. The air temperature ranged from 20°C to 37°C. The

minimum was recorded in February 2001 at SSII and maximum in October at SSI. The pattern of temperature fluctuation was more or less similar in all the three sampling stations. The temperature of water depends on the season and on the temperature of the ground with which it is in contact. The temperature of Torsa varied from 17.5° to 30°C. A maximum difference of 10-11°C between air and water temperature was observed in the month of October at all sampling stations. A minimum difference of 1°C between air and water temperature was observed at SSII in the month of February. Lower water temperatures were recorded in January and February at all stations. In general, the pH values of Torsa remained >8.0 in all months except the pre-monsoon months. We shall be discussing the implication of higher pH value of Torsa in conjunction with the total alkalinity value.

The conductivity of water depends upon the concentration of ions and its nutrient status and the variation in dissolved solid content is indicated by conductivity measurements. Torsa showed a range of 100 to 280µmhos/cm. The conductivity values of the post monsoon months nearly doubled the values obtained in pre-monsoon months at SSII and III. On the contrary, maximum conductivity value from SSI was obtained in January. A constant value of 120µmhos/cm was observed in pre-monsoon months at SSII. Minimum conductivity value was obtained in the month of June at all stations.

The suspended particles, soil, silt, decomposed or undecomposed organic matter, total dissolved solids as well as microscopic organisms etc. are the main source of turbidity in water, which always interferes with the penetration of light. The turbidity values ranged from 1.0 to 5.5 NTU in winter months. Turbidity in pre and post monsoon months was not studied as this parameter was lately introduced. A maximum value of 69 NTU was observed in May at SSII.

Total dissolved solids in Torsa were found to be low (32.0 to 46.9 mg/l) in March at all stations. The maximum value of 556mg/l was

observed in the month of May at SSI, while the highest values obtained from SSII and III were in the month of June.

The hardness of water reflects the nature of geologic formation with which the water is in contact. Total hardness value of Torsa ranged

Table - 1 : Average values of physico-chemical & microbiological features of water of Torsa River (1999-2001) at Hasimara (Sample Site-I)

Sl. No.	Physico-chemical characters	Pre-monsoon			Post-monsoon			Winter		
		Mar. 2000	May 2000 & 2001	June 2001	Sept. 1999	Oct. 2000	Nov. 2000	Dec. 2000	Jan. 2000 & 2001	Feb. 2001
01.	Air temperature ($^{\circ}\text{C}$)	27.0	27.6	29.0	32.6	37.0	26.0	26.0	23.0	27.5
02.	Water temperature ($^{\circ}\text{C}$)	18.5	22.3	25.0	25.5	26.0	21.0	20.5	19.0	20.0
03.	pH	7.1	7.4	8.0	8.5	8.6	8.6	8.3	8.1	8.7
04.	Conductivity (μ mhos/cm)	170	130	100	110	190	200	130	230	190
05.	Turbidity (NTU)	--	--	3.9	--	--	--	1.2	1.3	1.0
06.	TDS (mg/l)	46.9	556	101.3	--	--	--	225.6	206.7	88.0
07.	TSS (mg/l)	229	130.9	196.7	--	--	--	26.3	--	--
08.	Total hardness (mg/l)	--	65.2	36.7	--	74.0	86.0	102.0	38.4	42.4
09.	Ca-hardness (mg/l)	20.8	43.7	9.7	-	16.6	46.1	19.0	11.5	11.3
10.	Mg-hardness (mg/l)	-	21.5	27.0	-	57.4	40.0	83.0	26.9	31.1
11.	Alkalinity (mg/l)	-	-	117.1	-	-	-	13 4.4	-	115.0
12.	Chloride (mg/l)	-	-	6.5	-	-	-	-	5.8	9.9
13.	DO (mg/l)	8.1	7.5	6.7	7.5	7.5	8.2	8.1	8.5	8.2
14.	BOD (For 5 days at 20°C (mg/l)	0.75	1.13	0.7	0.2	1.26	0.8	1.3	1.3	0.8
15.	COD (mg/l)	2.0	-	1.5	6.4	6.6	4.8	0.8	2.4	3.2
16.	Free ammonia 'N' (mg/l)	17.0	17.3	15.2	21.7	8.8	36.2	15.5	18.2	61.2
17.	Albuminoid ammonia 'N' (mg/l)	0.31	0.32	0.02	0.51	0.24	1.2	0.49	2.1	3.3
18.	Nitrate 'N' (mg/l)	0.13	0.98	0.12	0.17	0.25	1.2	0.1	0.3	0.1
19.	Nitrite 'N' (mg/l)	0.006	0.008	0.003	0.002	0.007	0.004	0.001	0.004	0.007
20.	Dissolved phosphate (mg/l)	0.77	0.87	0.55	0.68	-	0.26	0.24	0.54	0.18
21.	Total phosphate (mg/l)	-	-	1.1	-	-	-	-	6.77	39.75
22.	Total phosphorus (mg/l)	-	-	0.35	-	-	-	-	2.18	12.8
Microbiological parameters										
01.	Heterotrophic count (CFU/ml)	-	9×10^4	9×10^3	5×10^4	1×10^6	1×10^4	-	4.5×10^4	-
02.	Total coliform (MPN/100 ml)	-	>1600	>1600	>1600	1100	>1600	>1600	100	-
03.	Fecal coliform (MPN/100ml)	-	1600	900	1600	-	900	1600	80	-
04.	Fecal streptococci (MPN/100ml)	-	11	240	70	-	50	70	-	-
-	Not done									

from 32 to 126 mg/l. This value range of the swift Torsa may be compared with mountainous Bhagirathi at Uttarkashi, Tehri and Deoprayag

where the values ranged from 27 to 71 mg/l, being minimum in high flow seasons and maximum in lean seasons (Gautam, 1990). Interestingly, in the

month of October, November and December the total hardness, values of Torsa were characteristically high and on average double, the

average value obtained in other months from all the sampling stations. Magnesium-hardness of Torsa ranged from 19.8 to 101.4 mg/l, which is

Table - 2 : Average values of physico-chemical & microbiological features of water of Torsa River (1999-2001) at Falakata (Sample Site-II)

Sl. No.	Physico-chemical characters	Pre-monsoon			Post-monsoon			Winter		
		Mar. 2000	May 2000 & 2001	June 2000 & 2001	Sept. 1999	Oct. 2000	Nov. 2000	Dec. 2000	Jan. 2000 & 2001	Feb. 2001
01.	Air temperature ($^{\circ}\text{C}$)	29.0	28.5	29.8	31.6	35.0	27.0	27.0	26.5	20.0
02.	Water temperature ($^{\circ}\text{C}$)	20.5	26.3	24.2	30.0	25.0	22.5	22.0	20.5	19.0
03.	PH	7.4	8.1	8.3	7.8	8.4	8.5	8.1	8.15	8.0
04.	Conductivity (μ mhos/cm)	120.0	120.0	120.0	264	220	270	190	165	190.0
05.	Turbidity (NTU)	-	69.0	-	-	-	-	1.5	1.1	1.1
06.	TDS (mg/l)	34.3	124.5	452.0	-	-	-	203.1	224.3	129.2
07.	TSS (mg/l)	249.0	124.5	129.4	-	-	-	23.1	--	--
08.	Total hardness (mg/l)	-	36.0	59.7	-	90.2	114.0	110.0	50.4	56.4
09.	Ca-hardness (mg/l)	14.4	15.5	29.8	-	19.5	55.7	23.5	13.4	14.9
10.	Mg-hardness (mg/l)	-	20.0	19.8	-	70.3	67.7	86.5	37.0	41.5
11.	Alkalinity (mg/l)	-	113.4	-	92	-	-	113.1	-	110
12.	Chloride (mg/l)	-	6.9	-	-	-	-	-	7.9	10.1
13.	DO (mg/l)	8.6	6.5	7.1	6.9	7.5	7.8	7.9	8.5	8.1
14.	BOD (For 5 days at 20°C (mg/l)	0.89	0.70	0.95	1.2	1.3	1.1	0.4	1.6	1.1
15.	COD (mg/l)	2.7	2.6	-	5.4	1.6	4.8	1.2	2.7	9.7
16.	Free ammonia 'N' (mg/l)	16.5	19.0	16.3	9.0	22.2	35.5	8.0	15.4	52.15
17.	Albuminoid ammonia 'N' (mg/l)	0.33	0.43	0.38	0.24	0.47	1.3	0.92	0.23	3.2
18.	Nitrate 'N' (mg/l)	0.20	0.13	1.67	0.29	0.22	1.9	0.17	0.44	0.09
19.	Nitrite 'N' (mg/l)	0.005	0.005	0.006	0.007	0.007	0.003	0.008	0.008	0.008
20.	Dissolved phosphate (mg/l)	0.77	0.30	0.21	-	0.87	0.42	0.30	0.72	0.208
21.	Total phosphate (mg/l)	-	3.78	-	-	-	-	-	4.47	3.52
22.	Total phosphorus (mg/l)	-	1.21	-	-	-	-	-	1.44	1.14
Microbiological parameters										
01.	Heterotrophic count (CFU/ml)	5×10^4	5×10^5	-	2×10^6	4×10^4	3×10^4	-	1.9×10^2	-
02.	Total coliform (MPN/100 ml)	1600	1600	-	1100	1600	500	240	130	-
03.	Fecal coliform (MPN/100ml)	1600	900	-	-	1600	300	50	80	-
04.	Fecal streptococci (MPN/100ml)	900	-	-	-	22	140	23	11	-

- Not Done

higher than the value reported for mountainous Bhagirathi (2-27 mg/l) (Gautam, 1990). In the

month of October, November and December, there was a characteristic increment of Mg-

hardness in Torsa (Table 1.1 to 1.3). This observation may be correlated with the dolomite mining in the dry season and subsequent washout in the river water.

Total alkalinity value of Torsa ranged from 82.0 to 134.4 mg/l, which is significantly high compared to the range of 16-60 mg/l in mountainous Bhagirathi stream as observed by

Table - 3 : Average values of physico-chemical & microbiological features of water of Torsa River (1999-2001) at Cooch Behar (Sample Site-III).

Sl. No.	Physico-chemical characters	Pre-monsoon			Post-monsoon			Winter		
		Mar. 2000	May 2001	June 2001	Sept. 1999	Oct. 2000	Nov. 2000	Dec. 2000	Jan. 2001	Feb. 2001
01.	Air temperature ($^{\circ}\text{C}$)	28.0	31.0	28.5	31.0	35.0	28.0	25.5	23.5	25.0
02.	Water temperature ($^{\circ}\text{C}$)	19.5	27.5	24.0	29.0	25.0	23.0	20.0	17.5	20.0
03.	pH	7.8	7.8	8.16	8.1	8.4	8.3	8.0	8.16	8.1
04.	Conductivity (μ mhos/cm)	125	150	110	225	280	270	190	140	170
05.	Turbidity (NTU)	-	16	-	-	-	-	1.5	1.0	5.5
06.	TDS (mg/l)	32.0	102.5	380	--	--	--	197.3	-	129.6
07.	TSS (mg/l)	202	650	128.8	--	--	--	21.6	-	-
08.	Total hardness (mg/l)	-	39.17	56.1	-	124.1	130	126	63.6	51.1
09.	Ca-hardness (mg/l)	20.84	15.2	32.3	-	23.5	16.5	24.8	16.8	13.2
10.	Mg-hardness (mg/l)	-	23.9	23.8	-	100.5	63.5	101.4	46.8	37.8
11.	Alkalinity (mg/l)	-	82.0	-	-	-	-	100.4	-	100.2
12.	Chloride (mg/l)	-	7.6	-	-	-	-	-	7.9	11.1
13.	DO (mg/l)	8.6	7.1	7.5	7.1	7.5	7.8	8.3	8.7	8.1
14.	BOD (For 5 days at 20°C (mg/l)	1.09	1.9	0.67	1.6	1.4	1.4	1.9	1.6	1.3
15.	COD (mg/l)	4.7	2.9	-	5.4	8.0	-	2.8	2.5	9.8
16.	Free ammonia 'N' (mg/l)	88.3	19.03	15.7	8.7	22.3	23.7	10.6	17.07	50.28
17.	Albuminoid ammonia 'N' (mg/l)	0.41	0.03	0.37	0.29	0.7	1.0	0.92	4.8	3.1
18.	Nitrate 'N' (mg/l)	0.20	0.12	1.05	0.29	0.22	2.2	0.14	0.09	0.09
19.	Nitrite 'N' (mg/l)	0.004	0.005	0.001	0.006	0.007	0.002	0.008	0.006	0.008
20.	Dissolved phosphate (mg/l)	0.61	0.41	0.24	-	1.1	0.46	0.38	0.20	0.21
21.	Total phosphate (mg/l)	-	3.6	-	-	-	-	-	4.47	4.43
22.	Total phosphorus (mg/l)	-	1.17	-	-	-	-	-	1.44	1.43
Microbiological parameters										
01.	Heterotrophic count (CFU/ml)	-	6.5×10^5	3×10^5	3×10^5	1.9×10^4	2.7×10^5	-	5.1×10^2	-
02.	Total coliform (MPN/100 ml)	-	1600	-	-	900	1600	1600	117	-
03.	Fecal coliform (MPN/100ml)	-	1600	-	-	300	1600	1600	117	-
04.	Fecal streptococci (MPN/100ml)	-	80	-	-	22	22	80	-	-

- Not Done

Gautam (1990). Total alkalinity is a measure of bicarbonates, carbonates and hydrates. The alkalinity of Torsa was found higher than the hardness value. Alkalinity and pH are the factors

in determining the amenability of the water to biological treatment (Manivasakam, 1980). It is explained that if the alkalinity is greater than hardness, it indicates the presence of basic salts-

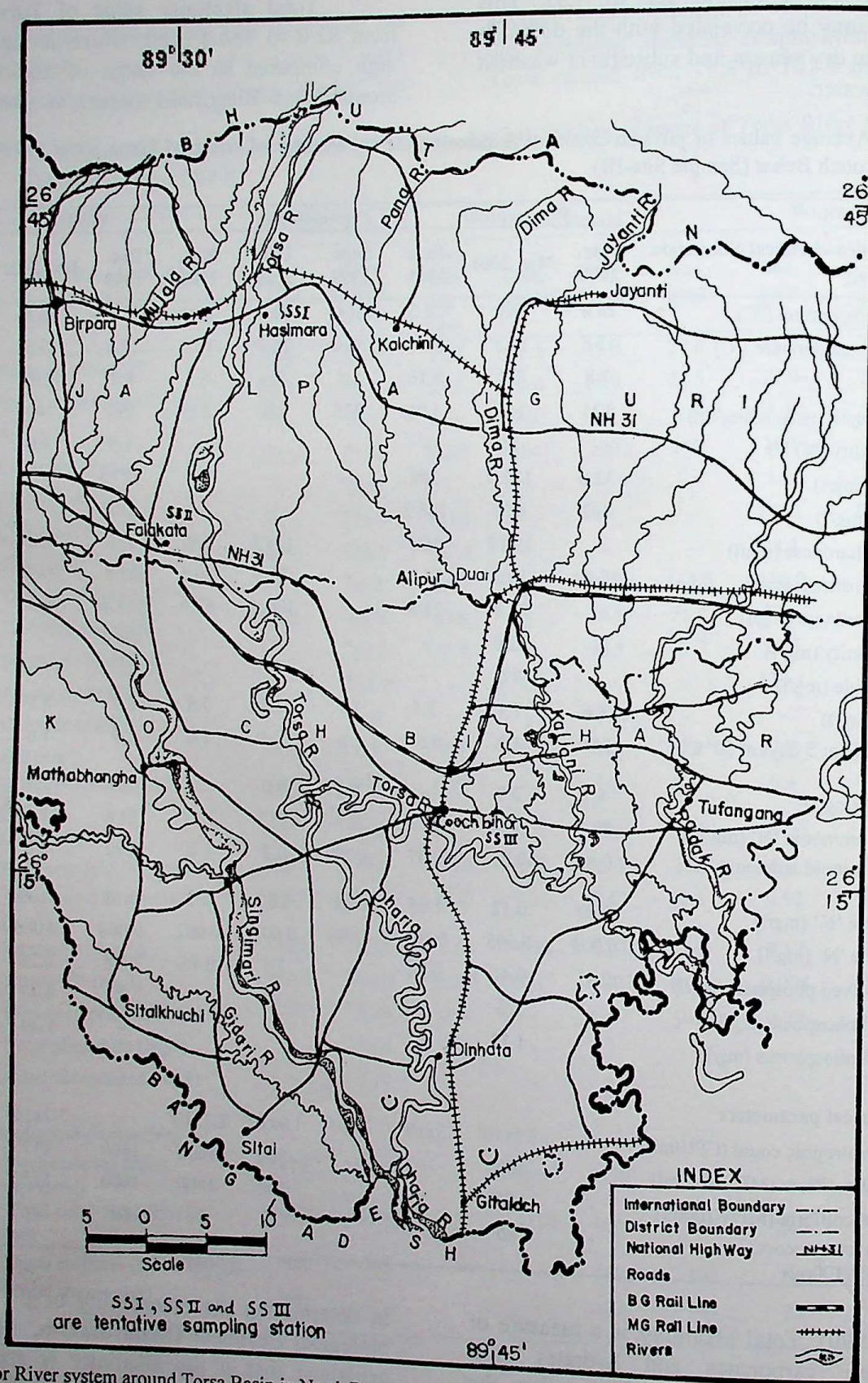


Fig. 1 : Major River system around Torsa Basin in North Bengal.

sodium and potassium in addition to those of calcium and magnesium.

Another parameter, that was introduced lately in our studies was chloride, which is the common anion found in water and sewage. Torsa contained chloride in the range of 5.8 to 11.1 mg/l. Chloride concentration was found relatively high in the month of February from all the stations. The concentration of chloride in Bhagirathi waters varied from 2.8 to 4.3 mg/l (Gautam, 1990).

During the period of investigation the minimum values of DO was recorded 6.5 and 6.7 mg/l at SSII (May) and SSI (June) respectively, while the maximum value of 8.7 mg/l was obtained in January at Sample site III. All the spots showed more or less similar pattern of variation in DO values having higher values during winter. Low temperature and aeration rate during winter was possibly responsible for increased amount of dissolved oxygen. Torrential nature of the river and its gradient may be held responsible for its average high value of DO throughout the course of this river. Bhagirathi at Uttarkashi, Tehri and Deoprayag yielded minimum DO value of 7.0 mg/l and maximum value of 10.9 mg/l (Gautam, 1990).

The BOD value of SSIII was in the range of 1.09 to 1.9 mg/l except for the month of June, which showed 0.67 mg/l. The BOD value fluctuated between 0.7 to 1.6 mg/l at SSII except December, which showed a value of 0.4 mg/l. For SSI the BOD values were in the range of 0.7 to 1.3 mg/l except September that showed 0.2 mg/l. BOD value of Torsa did not exceed 2.0 mg/l. The BOD values of mountainous Bhagirathi fluctuated from 1.5 to 6.9 mg/l (Gautam, 1990). Even within the narrow range, BOD values of Torsa were comparatively higher during winter months at all stations which may be due to low dilution capacity of river during winter. To explain the overall low BOD values in other months, it may be argued otherwise that increased temperature, high flow and increased sediment load reduced the BOD concentration during summer and post

monsoon months in river water (Pyatkin and Krivoshein, 1980; Goltermann *et al.*, 1983).

Maximum COD value of 9.7 to 9.8 mg/l was recorded in the month of February from SSII and III. Maximum COD value from SSI was recorded in the post monsoon months (September and October). In case of mountainous Bhagirathi, the COD fluctuated between 5.25 to 11.53 mg/l (Gautam, 1990).

The free ammonia content was many times the content of albuminoid ammonia in the River Torsa. When interpreting the results, the free ammonia and albuminoid ammonia should be considered together, since the relative proportion is more important than the actual quantities. Hence, Torsa water, which contains more free ammonia than albuminoid ammonia, the water, may be suspected of polluted with sewage. The presence of more than traces of ammoniacal nitrogen in river water (which is used as drinking water source) is undesirable. Maximum value of 88.3 mg/l free ammonia was obtained in the month of March from SSIII. All the sampling stations showed higher level of free ammonia in the month of February (50 to 61 mg/l). The ratio of free ammonia to albuminoid ammonia was found much higher in the pre-monsoon months (range, 42 to 761) than the winter (range, 4 to 67). The lowest ratio of four was observed in January from SSIII. A rough guide with regard to the ammonia is that if the albuminoid ammonia content is 0.08 mg/l the free ammonia content should not exceed 0.05 mg/l. If it exceeds the limit, manurial pollution may be suspected (Manivasakam, 1980).

Nitrites are generally formed in water due to bacterial action on ammonia and organic nitrogen. Since nitrites are readily oxidized to nitrates, they are seldom present in significant concentration in surface waters. Nitrite was present in the range of 0.001 to 0.008 mg/l. On the other hand, nitrate content of Torsa River was in the range of 0.09 to 2.2 mg/l. The level of nitrates in mountainous Bhagirathi was observed between 0.001 and 0.99 mg/l (Gautam, 1990). The low level of nitrate as well as nitrite in

comparison to high level of ammonia nitrogen indicates that the nitrogenous organic matter is undergoing oxidation or nitrification and that the process is far from being complete.

Interestingly, the dissolved phosphate content of Torsa River did not exceed 1.0 mg/l while total phosphate and total phosphorus content reached its maximum value of 39.75 mg/l and 12.8 mg/l respectively in the month of February from SSI.

Fecal pollution is a major concern for many rivers of North Bengal where it can originate from human sources and non-human sources (our unpublished observation). Its impact can degrade water quality and restrict its use for drinking and recreational activities. River Torsa receive fecal pollution from a variety of sources, including humans, cattle and wild life. The fecal coliform *Escherichia coli* has been used as an indicator of human enteric pathogens for many years (Goldreich, 1966). However, it is now well established that *Escherichia coli* is not limited to humans but also exists in the intestines of many other warm-blooded animals (Orskov and Orskov, 1981). Consequently, its presence in water is not specific to human sources of pollution. It is, therefore, important to know whether fecal pollution originates from human or non-human source in order to properly assess the risk. There have been attempts to develop methods that differentiate the sources of fecal pollution. Initially, the ratio of fecal coliforms to fecal streptococci was proposed where a ratio of >4.0 would indicate human source pollution, whereas a ratio of <0.7 would indicate non-human source pollution (Goldreich and Kenner, 1969). Ratios between 0.7 and 4.4 usually indicate wastes of mixed human and animal sources (APHA, 1989). In the present discussion, we would not like to consider those ratios where fecal streptococcus contents were found below 100/100 ml in order to minimize misinterpretation of ratios. The ratio of fecal coliform to fecal streptococci in Torsa ranged from 1.77 to 3.75. The maximum and minimum fecal coliform : fecal streptococci value was obtained in the month of June and March at

SSI and SSII respectively. SSI (Hasimara), which is just the downstream of Phuntsholing and Jaigaon settlement, receives fecal matter of human origin more than SSII. Falakata (SSII) being located after Jaldapara Wild Life Sanctuary, indicates fecal pollution from wastes of mixed human and animal sources in the month of March and November. Total coliform and fecal coliform count was found highest and the least in the month of March and January respectively from all sampling stations.

As it is not possible to recover all viable bacteria in a water sample with a single procedure, we have attempted standard plate count to determine the density of aerobic and facultative anaerobic heterotrophic bacteria in Torsa water. Heterotrophic bacterial load was found maximum (2×10^6 CFU/ml) in the post monsoon month of September at SSII. SSI and III record its maximum load of 1×10^6 and 3×10^5 CFU/ml in the month of October and September respectively. Low heterotrophic count (1.9 to 5.1×10^2 CFU/ml) was observed in January at SSII and III. It is also important to note that the water temperatures at the respective sampling station(s) was also at their highest in the respective month exhibiting highest heterotrophic bacterial load. It was also suggested by previous authors that higher water temperature induced the growth of bacteria, which resulted in increased metabolic activity, while low temperature reduced it (Pyatkin and Krivoshein, 1980). Another interesting observation was that when maximum heterotrophic bacterial load of water sample is at respective sampling stations (so far recorded), the free ammonia content detected was the least (See Table 1.1 to 1.3).

The major characteristics in chemical data are high pH, high alkalinity, high magnesium hardness and high free ammonia concentration and corresponding values of albuminoid ammonia concentration suggests high sewage pollution from catchment localities, hence sewage treatment planning in the catchment localities is suggested. High pH, alkalinity and magnesium hardness values indicate the soil erosion due to

excessive mining of dolomites in its catchment area. This study will, therefore, be useful in determining its suitability for different purposes in this region. It may also serve as the database for further studies on this river.

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Gender differences in the metabolism of benzene, toluene and trichloroethylene in rat with special reference to certain biochemical parameters.

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Abstract : *Metabolites viz. phenol, hippuric acid and total trichloro compounds of benzene, toluene and trichloroethylene respectively were estimated in the urine samples of male and female rats after exposure for a period of 30 days. The results exhibited higher metabolism in female rats than the male rats. Their metabolism might be regulated by cytochrome P₄₅₀ isozymes in a gender specific manner. However, sex differences in the activity of glutathione-S-transferases of the liver have also been found to determine their toxicity. Results have been discussed with quantitative profiles of other enzymes established in the liver of male and female rats.*

Key words : *Benzene, Toluene, Trichloroethylene, Phenol, Hippuric acid, Total trichloro compounds, Glutathione-S-transferases.*

Introduction

Industrial solvents comprise a large family of organic compounds to which man may be exposed during their manufacture, handling and use. Benzene, toluene and trichloroethylene are the three most important agents used in many industries as solvents (Paustenbach *et al.*, 1993, IARC 1995, Suzuki *et al.*, 1983). Several sources contribute to the exposure of man to benzene, toluene and trichloroethylene. Benzene is a constituent of gasoline, automobile exhaust and tobacco smoke (Wallace 1990). In humans, long term exposure to benzene is associated with hematotoxicity, genotoxicity and acute myelogenous leukemia (Aksoy 1989). There are evidences in humans, which indicate that lung cancer may result from benzene exposure (Aksoy, 1980, Yin *et al.*, 1996). The sniffing glue is a major source of high exposure to toluene (Ehyai and Freeman, 1983). Inhalation of toluene achieved by sniffing can be more than 50 times the current occupational exposure limits of 100 ppm (Press *et al.*, 1967). The workers from printing and paint manufacturing industries are known to be exposed to approximately 100-365 ppm toluene (Morata *et al.*, 1993). In USA alone, more than 400,000 workers are exposed to trichloroethylene (NIOSH, 1994). In a survey

report, concentration of trichloroethylene was estimated to be approximately 1 ppb in the troposphere of many industrialized cities (WHO, 1985).

Several factors like age, diet, alcohol intake and phenobarbital play a major role in toxicity of these solvents (Ikeda *et al.*, 1971., Kumar *et al.*, 1993, Rana *et al.*, 1999). Reports indicated sex-differences in the toxicity of a few solvents (Poon *et al.*, 1994., Nomiyama *et al.*, 1969). Toxicokinetics of benzene, toluene and trichloroethylene have revealed that they are metabolized into phenol, hippuric acid and trichloro compounds respectively and excreted in the urine. However, sex differences on their excretion are not known. Therefore, influence of gender differences on the excretion of their metabolites was studied. Differences in conjugating mechanisms, if any, have been studied through glutathione-S-transferase activity in the liver.

Materials and Methods

Healthy male and female Wistar rats (110 \pm 20 gm) were procured from animal facility of Jamia Hamdard, New Delhi, as and when required and maintained under standard laboratory

conditions (room temp $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$, relative humidity $60 \pm 10\%$) in the animal house of Department of Zoology, C.C.S. University, Meerut. Each rat was housed in a polypropylene cage on rice husk bed and offered commercial food pellets (Golden Feeds, New Delhi) and water *ad libitum*. These experiments were done after the approval of institutional animal ethical committee and Ministry of Social Justice and Empowerment, Govt. of India.

Rats of both sexes were divided into six groups of five rats each. Rats were treated with predetermined (Rana and Kumar, 1993., Rana and Gupta, 1999) sublethal doses of benzene, toluene and trichloroethylene (0.25 ml/100gm body weight) respectively on each alternate day for 30 days. Rats treated with olive oil served as controls. Solvents and olive oil were injected intraperitoneally. A daily record of food and water consumed and the body weight of each rat were also maintained. After the scheduled treatments, urine samples were collected through metabolic cages and rats were starved overnight and sacrificed by light ether anesthesia, the next morning.

The liver and kidney were carefully removed and weighed on an electronic balance (Sartorius, Germany). Specific gravity of the urine samples was determined by an urinometer (Atago Company Limited, Japan). Creatinine in the urine samples was estimated using alkaline picrate method of Toro and Ackerman (1975).

Phenol, a metabolite of benzene was estimated by the method of Danis (1951). Pure liquid phenol was used as the standard.

Hippuric acid, a metabolite of toluene was determined by the direct colorimetric method of Ogata and Hobara (1979). Pure crystalline hippuric acid (Merck, Germany) was used as the standard.

Total trichloro compounds, the metabolites of trichloroethylene in urine samples were estimated following the method of Ogata and co-workers (1987) using phosphate buffer

containing 500 units of β -glucuronidase from *Helix pomatia* (Sigma, USA).

Glutathione-S-transferases were estimated by employing the method of Habig *et al.* (1974).

All other chemicals used in this study were of highest purity available in the market.

Student's 't' test was applied for statistical inferences.

Results and Discussion

Presence of the metabolites of benzene, toluene and trichloroethylene in the urine samples of control rats, though in traces, indicate the ubiquity of these compounds in the environment. They might have entered the animal systems through food, water and/or air, detoxicated and eliminated. However, sex differences were recorded even in control rats, for their metabolites viz. phenol, hippuric acid and total trichloro compounds. In benzene treated rats, values on parameters like specific gravity, creatinine, phenol and glutathione-S-transferase activity were higher in female rats than the male rats. Almost similar results were recorded in toluene and trichloroethylene treated rats. These observations show that female rats are able to metabolize these solvents better than the male rats. In other words, these solvents are expected to be more toxic in male rats than the female rats (Tables 1 and 2).

A primary goal of this study was to compare the metabolism of benzene, toluene and trichloroethylene in male and female rats. It is widely accepted that the effect of a xenobiotic is related to its concentration at the site of action (Levy and Gibaldi, 1972). The sex differences in the susceptibility if any, should largely be attributed to the differences between the concentration of the xenobiotic at the site of action attained in the male and the female, which in turn is controlled, by absorption, distribution and excretion of the chemical.

The modifying role of sex in response to pharmacological agents and toxic chemicals has a specific metabolic background connected with the

control of metabolizing enzymes by estrogenic and androgenic hormones. Hirokawa (1955) concluded that the follicular hormone might play

a major role in the manifestation of sex differences in the susceptibility to benzene. Available literature

Table - 1 : Observations on benzene, toluene and trichloroethylene treated male rats

Observations	Control	Benzene	Toluene	Trichloroethylene
Body weight (gm)	120 ± 1.6 (I) 210 ± 4.6 (F)	120 ± 1.9 (I) 142 ± 3.8 (F)*	112.5 ± 1.0 (I) 145 ± 1.3 (F)*	113 ± 1.0 (I) 200 ± 4.1 (F)*
Liver weight (gm)	6.0 ± 0.6	3.5 ± 0.03*	4.6 ± 0.17*	6.5 ± 0.1 ^{NS}
Liver-body weight ratio	2.8 ± 0.01	2.4 ± 0.01*	3.1 ± 0.03 ^{NS}	3.3 ± 0.06 ^{NS}
Kidney weight (gm)	1.4 ± 0.10	1.4 ± 0.10 ^{NS}	1.3 ± 0.08 ^{NS}	1.4 ± 0.10 ^{NS}
Kidney-body weight ratio	0.6 ± 0.01	0.98 ± 0.01*	0.90 ± 0.1*	0.70 ± 0.01 ^{NS}
Specific gravity of urine	1.023 ± 0.001	1.032 ± 0.001 ^{NS}	1.028 ± 0.02 ^{NS}	1.022 ± 0.001 ^{NS}
Creatinine (mg/l)	240 ± 0.01	230 ± 0.07 ^{NS}	200 ± 0.01*	220 ± 0.02 ^{NS}
Phenol (mg/l)	147 ± 0.01	680 ± 6.8*	-	-
Hippuric acid (mg/l)	650 ± 1.7	-	675 ± 1.8*	-
Total trichloro compounds (mg/l)	0.51 ± 0.01	-	-	2.07 ± 0.01*
Liver glutathione-S-transferase (n moles/NADPH/min/mg protein/cytosol)	0.700 ± 0.14	0.510 ± 0.01*	0.321 ± 0.12*	0.460 ± 0.12*

Results are expressed as mean ± S.E (n=5)

* values are significantly different from controls (P<0.05).

I denotes-initial; F denotes-final

NS - non significant

Table - 2 : Observations on benzene, toluene and trichloroethylene treated female rats

Observations	Control	Benzene	Toluene	Trichloroethylene
Body weight (gm)	115 ± 1.8 (I) 205 ± 3.8 (F)	115 ± 2.6 (I) 135 ± 1.0 (F)*	120 ± 1.6 (I) 165 ± 1.8 (F)*	120.5 ± 1.9 (I) 115 ± 1.8 (F)*
Liver weight (gm)	6.2 ± 0.1	3.1 ± 0.02*	5.0 ± 0.63*	4.5 ± 0.16*
Liver-body weight ratio	3.0 ± 0.01	2.3 ± 0.01 ^{NS}	3.0 ± 0.01 ^{NS}	3.9 ± 0.01 ^{NS}
Kidney weight (gm)	1.5 ± 0.95	1.3 ± 0.02 ^{NS}	1.2 ± 0.08 ^{NS}	1.1 ± 0.10 ^{NS}
Kidney-body weight ratio	0.7 ± 0.008	0.96 ± 0.01*	0.74 ± 0.01 ^{NS}	1.3 ± 0.02*
Specific gravity of urine	1.028 ± 0.02	1.038 ± 0.001*	1.030 ± 0.001 ^{NS}	1.029 ± 0.001 ^{NS}
Creatinine (mg/l)	220 ± 0.02	270 ± 0.04*	220 ± 0.02 ^{NS}	350 ± 0.01*
Phenol (mg/l)	152 ± 0.03	762 ± 5.1*	-	-
Hippuric acid (mg/l)	625 ± 1.7	-	750 ± 1.7*	-
Total trichloro compounds (mg/l)	0.77 ± 0.07	-	-	2.65 ± 0.01*
Liver glutathione-S-transferase (n moles/NADPH/min/mg protein/cytosol)	0.692 ± 0.01	0.620 ± 0.16*	0.748 ± 0.14*	0.962 ± 0.22*

Results are expressed as mean ± S.E (n=5)

* values are significantly different from controls (P<0.05).

I denotes-initial; F denotes-final

NS - non significant

shows that female rats (Ikeda, 1964), rabbits (Hirokawa, 1955) and humans (Hirokawa, 1960) have a higher degree of susceptibility than the males to hematopoietic disorders of benzene.

However, no satisfactory explanation is available for the sex differences. The sex and strain differences have been observed for phenobarbital inductions (Larsen *et al.* 1994a., Larsen *et al.*

1994b., Larsen and Jefcoate, 1995). Metabolism of toluene is a CYP₄₅₀ catalyzed reaction that occurs primarily in the liver. Regulation of CYP₄₅₀ in a gender specific manner has been reported by Ikegwuonu (1996). Gender differences in the toxicity of methanol and toluene have been reported in rats (Poon *et al.*, 1994). A number of solvents including benzene, toluene and trichloroethylene may induce xenobiotic metabolizing enzymes e.g. CYP2E1 (Conney, 1968; Nebert *et al.*, 1978). Sex differences in these enzyme activities have been observed. Ethyl benzene treatment induced CYP2B1 and CYP2B2 to a greater extent in male than in female rats and CYP2E1 only in female rats (Sequeria *et al.*, 1992). The glycol ethers 2-methoxy ethanol and 2-ethoxy ethanol induced liver alcohol dehydrogenase in male rats only while no induction was observed in female rats (Conney, 1968). Dekant and coworkers (1995) also reported sex, organ and species specific bioactivation of chloromethane by cytochrome P₄₅₀2E1. They attributed these differences to sex differences in liver microsomal oxidations.

Above discussion puts major emphasis on sex differences observed in CYP₄₅₀. However, only a part of these organic solvents is converted into epoxides by CYP₄₅₀ while rest is detoxified through GSH conjugation catalyzed by GSH-S-transferase. Thus, a study on GSH-S-transferases might be helpful in understanding gender differences in solvent toxicity. Glutathione conjugates commonly appear in urine as mercapturic acids or cysteine conjugates (Chasseaud, 1979). Mercapturic acids are the end products of the conjugation reaction of GSH with an electrophile and are readily excreted in the urine.

The glutathione-S-transferases form a large family of enzymes with overlapping substrate specificity. Ten isoenzymes have been reported in rat liver alone and this constitute more than 5% of the total protein that can be extracted from the liver of rats (Jakoby, 1978). Inducers such as phenobarbital double the value in rat as does thyroidectomy (Reyes *et al.*, 1971). Aside

from its ability to catalyze, the enzyme may be looked upon as a binding protein. This binding and possibly storage function is believed to be a major role of glutathione-S-transferases and therefore, they are called as ligandins. There appear to be as many "ligandins" as these ligandins might occur in both rat and human (Ketley *et al.*, 1975). Intrinsic and broad binding powers of this series of enzymes might be another explanation for gender differences. Midgut of female crayfish *Procambarus clarkii* had higher glutathione-S-transferase activity than the male crayfish (Almar *et al.*, 1987). Quantitative profiles established in the liver of male and female rats for other enzymes viz. glucose-6-phosphatase, glucose-6-phosphate dehydrogenase and malic enzyme as well as those of glycogen (Teutsch, 1981) indicating sex related difference support present observations. Present results suggest that a study on sex differences should always be a part of ecotoxicological investigations and studies on industrial health.

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Response of sugarcane to treated wastewater of oil refinery.

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Abstract : The crop of sugarcane (*Saccharum officinarum*) was grown at the agricultural farm of the Mathura Oil Refinery in a simple randomized block design. The experimental plots were irrigated with ground water (GW) or treated wastewater (TW) as and when required. The plants gave better response to the latter than the former. The quantity of the required nutrients was comparatively more in TW than GW. The soil receiving wastewater did not show any significant change in its physico-chemical characteristics. The soil accumulated all the heavy metals but the plant samples receiving TW only exhibited the presence of Ni, Pb and Zn whose values are far below the permissible limits.

Key words : Growth, Soil, Sugarcane, Wastewater.

Introduction

The increase in the number of industrial installations is very much associated with a substantial increase in the quantum of the liquid waste. These, so called, treated wastes are being indiscriminately discharged either directly to the open land or into the nearby water body which in turn becomes the source of water, required to irrigate the crops. Though, the soil is naturally characterized with a great degree of filtration and decomposition capacity even then a sufficient quantity of undesired substances may be retained by it as pollutant. One of the major components is the nutritional status whose rate of leaching and precipitation depends on the nature of the wastewater and the soil type. The plants, therefore, supported by such a soil express varied response depending on the plant type. The irrigation with municipal wastewater and the effluents from paper, sugar, brewery and textile factories improved the performance of various crops without affecting the soil properties (Day *et al.*, 1975; Bole and Bell, 1978; Rajannan and Oblisami, 1979; Ajmal and Khan, 1983, 1984, 1985). Moreover, certain short duration crops have also given positive response to the treated

wastewater of the oil refinery (Samiullah *et al.*, 1994; Aziz *et al.*, 1999; Hayat *et al.*, 2000).

The present study was undertaken with a difference from earlier studies that the long duration sugarcane crop was irrigated with the treated wastewater of the oil refinery more regularly during its extended growth period than short duration crops. Therefore, it may give a better picture about the impact of the wastewater on the biological produce and the soil characteristics.

Materials and Methods

The Mathura Oil Refinery is situated on National Highway No.2, about 10 km away from Mathura City towards Agra at the latitude of 27°-22N', longitude 77°-40'E and altitude 174m above MSL.

A piece of land, bounded by wastewater drain, was developed into an experimental farm for the purpose of the present trials. In a simple randomized field experiment, a uniform dose of N₇₅ P₆₀ K₆₀ was applied at the time of planting. Additional quantity of 75 kg N was also given as top dressing. The sources for N, P and K were urea, mono-calcium superphosphate and muriate

of potash, respectively. Cuttings of sugarcane (*Saccharum officinarum* cv. CoLk 8102) were planted in experimental plots of 25 m² and were irrigated with treated wastewater (TW) of the refinery or the ground water (GW). During the entire period of growth, the crop received six irrigations. At each irrigation, every plot received 2500 liters of TW or GW. The flow was measured by using a 90° V-notch weir box and the formula $Q = 1417 H^{2.5}$, where Q is the discharge (liters S⁻¹) and H is the height of water over the apex of the notch.

Samples of the irrigants (TW and GW) were collected just before irrigation. Four to five soil samples were collected from each plot at a depth of about 0-15 cm before planting and after harvesting the crop. Both irrigants and soil samples were analysed for various important physico-chemical properties (APHA, 1995; Ghosh *et al.*, 1983).

The plant fresh weight, number of nodes and average node length were studied at 50, 100, 150, 200, 250, 300 and 350 days, after planting.

Yield characteristics (cane length, cane girth and weight of 10 canes) and cane yield ton ha⁻¹ were studied, at harvest of the crop.

For determining heavy metals, the samples of soil, irrigants, leaves and sugarcane juice were digested in aqua regia (Vanloon and Lichwa, 1973) and analysed using a GBC 902 double beam atomic absorption spectrophotometer. The data for growth yield characteristics and cane yield was statistically analysed as described by Gomez and Gomez (1984).

Results and Discussion

It is self evident from the physico-chemical characteristics (Table 1) of ground water (GW) and treated wastewater (TW), employed to irrigate the crop of sugarcane, that the two differed significantly in most of the characteristics, except the pH, which was somewhat comparable with

Table - 1 : Irrigation water quality (all determinations given as ranges in mg l⁻¹ or as specified)

Parameters	Ground water	Treated wastewater
pH	7.5-7.9	7.4-8.1
E.C. (ds m ⁻¹)	0.59-0.76	0.94-1.26
HCO ₃ ⁻	118-135	148-215
Cl ⁻	75-110	135-160
Ca ⁺²	18-23	44-60
Mg ⁺²	14-35	18-60
K ⁺	4-11	16-18
Na ⁺	25-34	55-63
PO ₄ ⁻³	0.15-0.19	0.23-0.59
SO ₄ ⁻³	68-85	88-97
NO ₃ ⁻	0.85-1.40	0.95-1.80
COD	20-25	42-48
TDS	559-930	700-975

COD = Chemical oxygen demand

TDS = Total dissolved solid

each other. Treated wastewater exhibited higher electrical conductivity (EC), chemical oxygen demand (COD) and the level of all the ions, compared with GW. High Ca⁺⁺ and Mg⁺⁺, in TW, are not only expected to benefit the plant growth but will also be helpful in maintaining the proper

structure of the soil (Table 2). Similarly, an elevated level, which is below the toxic limits, of sodium (63 mg l⁻¹) and TDS (975 mg l⁻¹), is considered suitable for the growth of all types of the crops. However, TW retained a significant quantity of most of the heavy metals (Fig. 1), even

after best possible treatment, on being compared again with the GW. The presence of these ions was

Table - 2 : Physico-chemical characteristics of soil before sowing and after harvest of the sugarcane crop, irrigated with ground water and treated wastewater (all determinations in mg l^{-1} or as specified)

Parameters	Ground water		Treated wastewater	
	Before sowing	After harvest	Before sowing	After harvest
PH	7.60	7.80	7.90	8.20
E.C. (dsm^{-1})	0.75	0.79	0.85	0.98
HCO_3^-	185	193	196	199
Cl^-	35.50	38.10	49.75	60.63
Ca^{+2}	29.30	31.14	20.45	39.45
Mg^{+2}	19.23	23.86	29.18	39.11
K^+	14.00	16.00	19.00	20.00
PO_4^{-3}	0.79	0.86	0.84	0.93
Na^+	29.15	30.85	31.93	41.15
NO_3^-	1.10	1.20	1.34	1.38
Organic carbon (%)	0.39	0.48	0.45	0.53
Organic matter (%)	0.67	0.82	0.77	0.96
CEC	3.30	3.46	4.04	4.34
meq/100g				
TDS	779.0	789.0	819.0	827.0

CEC = Cation exchange capacity

TDS = Total dissolved solid

Table - 3 : Effect of ground water (GW) and treated wastewater (TW) on fresh weight, number of nodes and average node length of sugarcane at different stages of growth.

Treatments	Days of sampling						
	50	100	150	200	250	300	350
	Fresh weight (kg plant^{-1})						
GW	0.105	0.148	0.306	0.444	0.790	1.150	1.390
TW	0.136	0.173	0.375	0.565	0.885	1.450	1.593
C.D. at 5 %	0.030	0.021	0.056	0.081	0.065	0.113	0.105
	Number of nodes plant^{-1}						
GW	3.31	3.51	6.20	13.75	16.76	20.30	22.49
TW	3.40	3.66	6.63	14.62	17.93	21.40	24.15
C.D. at 5 %	N.S.	N.S.	0.25	0.41	0.36	0.51	0.69
	Average node length (cm)						
GW	10.32	10.89	14.04	10.15	10.53	10.08	9.10
TW	10.53	11.94	15.09	10.88	10.96	11.09	10.36
C.D. at 5 %	N.S.	0.31	0.45	0.21	N.S.	N.S.	0.13

C.D. = Critical difference

N.S. = Non-significant

felt when the analysis of the soil irrigated with TW or GW was compared (Fig.2). Therefore, the

presence of higher level of these essential elements in the soil, irrigated with TW, is simply

an expression of their relative values in the water itself. However, the values are comparable with those reported earlier (Hayat *et al.*, 2000) but are in the limits reported to be toxic by Krishna Murti and Vishwanathan (1991).

The wastewater during the course of its chemical treatment is enriched with certain essential elements (Table 1), which make it highly suited for the growth of the plants. It, therefore, enhanced the cane fresh weight by 29.5, 16.8,

Table - 4 : Effect of ground water (GW) and treated wastewater (TW) on cane length, cane girth, weight of 10 canes and cane yield of sugarcane at harvest.

Treatments	Cane length (cm)	Cane girth (cm)	Weight of 10 canes	Cane yield (ton ha ⁻¹)
GW	243.95	2.22	13.93	94.65
TW	247.35	2.29	14.86	101.35
C.D. at 5 %	2.65	N.S.	0.65	5.75

C.D. = Critical difference

N.S. = Non-significant

Table - 5 : Heavy metal content ($\mu\text{g g}^{-1}$) in leaves and juice of sugarcane irrigated with ground water (GW) or treated wastewater (TW).

Heavy metals	Leaves		Sugarcane juice	
	GW	TW	GW	TW
Cd	N.D.	N.D.	N.D.	N.D.
Cr	N.D.	N.D.	N.D.	N.D.
Cu	N.D.	N.D.	N.D.	N.D.
Ni	N.D.	0.01	N.D.	0.02
Pb	N.D.	0.01	N.D.	0.01
Zn	N.D.	0.11	N.D.	0.13
Mn	N.D.	N.D.	N.D.	N.D.

N.D. = Non-detectable

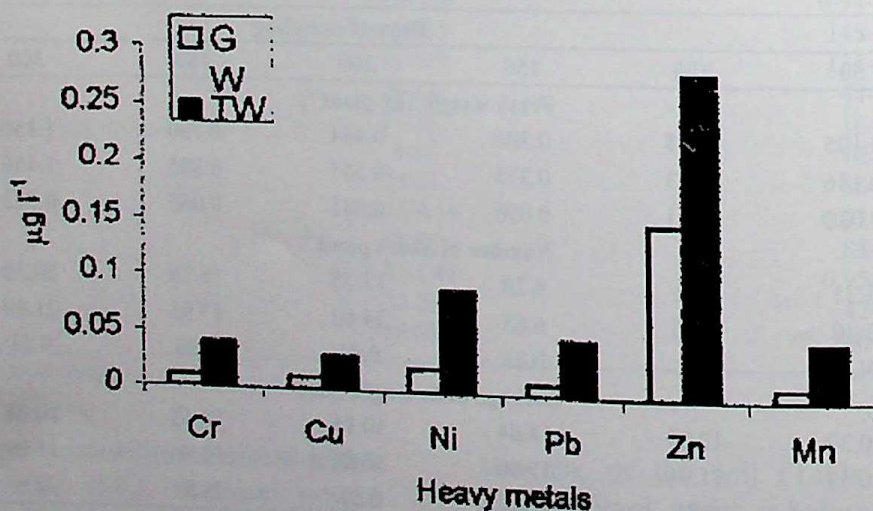


Fig. 1 : Heavy metal content in ground water (GW) and treated wastewater (TW).

22.5, 27.2, 12.0, 26.0, 14.6 % (Table 3), number of nodes per cane by 6.9, 6.3, 6.9, 5.4, 7.4 % (Table 3) and average node length by 9.6, 7.5,

7.2 and 13.8 % (Table 3) at various stages of growth, over those received GW. A cumulative effect of the above characteristics was noticed on

Response of sugarcane to treated wastewater.

cane length, weight of 10 canes and the cane yield (Table 4) increasing their values under TW by

1.4, 6.7 and 7.0 %, over the GW, respectively. Moreover, the wastewater from municipal waste

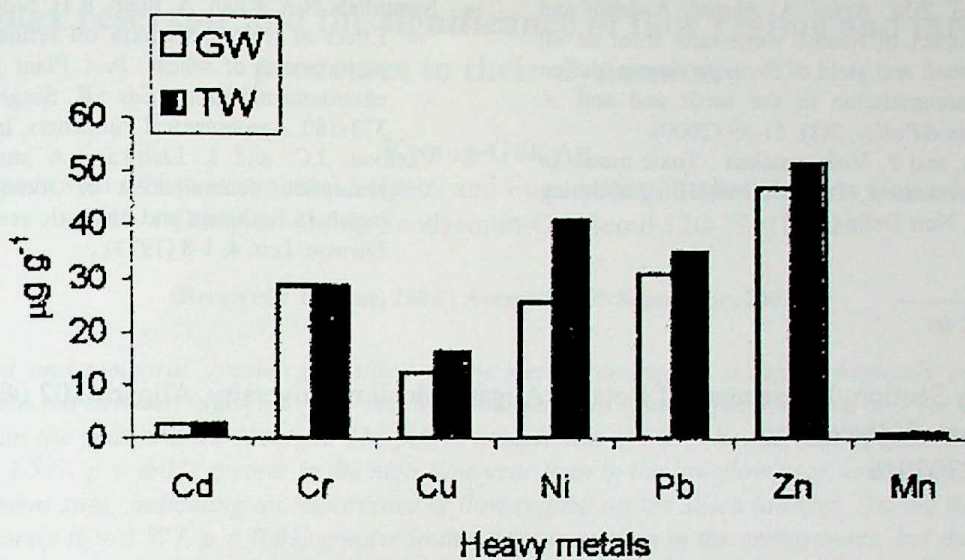


Fig. 2 : Heavy metal content in soil irrigated with GW or TW.

(Day *et al.*, 1975), effluent from paper (Rajannan and Oblisami, 1979), sugar (Ajmal and Khan, 1983), brewery (Ajmal and Khan, 1984), textile (Ajmal and Khan, 1985) industries and that from the oil refinery (Samiullah *et al.*, 1994; Aziz *et al.*, 1999; Hayat *et al.*, 2000) is reported to have generated economical response in short duration crops.

Most of the heavy metals in the leaves and juice of sugarcane are not traceable but Ni, Pb and Zn are present in the plants irrigated with treated wastewater, whose values are very much below the permissible limits (Table 5).

It may therefore, be suggested that the treated wastewater released from the Mathura Oil Refinery into the drain for irrigation purpose fulfills the criteria of an irrigant as laid down by Krishna Murti and Vishwanathan (1991). It suits the plants because of its richness in most of the essential elements, required for plant growth. Moreover, the soil characteristics are also not damaged to a significant state by its repeated application and heavy metal accumulation in soil and crop produce are under the permissible limit.

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Longitudinal and seasonal variations of epilimnetic silica in a morphologically complex reservoir and the significance of flow regime and internal processes to their dynamics.

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Abstract : Spatial and temporal dynamics of silica (SiO_2) were examined in a morphologically complex reservoir, based on data collected between high-flow year and low-flow year. SiO_2 averaged 3.4 mg/L and varied from 0.1 to 9.7 mg/L depending on the year and the location. The paired sample test of SiO_2 showed that in mainstem sites, SiO_2 was significantly ($t = 3.577$, $p < 0.01$) greater in the high-flow year than in the low-flow year, and this pattern was similar to that of embayment sites, indicating an importance of flow regime on the silica loading. During the high-flow year, SiO_2 was significantly ($t = 3.577$, $p < 0.01$) greater in the mainstems than in the embayments, but during the low-flow year, there was no statistical difference between the two reaches. SiO_2 showed a distinct longitudinal decline from the headwaters to the dam in the high-flow year, and it was modified by the plunging of metalimnetic density current in the mid-lake reach. Seasonal fluctuation of SiO_2 was influenced by internal nutrient cycling and diatom populations. Dominant phytoplankton abundance had an inverse relation between the two algal populations of bluegreens and diatoms during August-December of the low-flow year. In other words, bluegreen algae dominated at the low SiO_2 (< 2.5 mg/L) during the summer period of the low-flow year, whereas diatoms dominated with the increase of SiO_2 in fall overturn. Overall results suggest that increase of silica in this system is primarily regulated by interannual flow regime, but the internal loading during fall overturn and biological up-take by seasonal growth of diatom community were also considered as an important process controlling the input of silica.

Key words : Silica, Flow regime, Longitudinal gradient, Diatoms, Water residence time.

Introduction

During the past three decades, eutrophication phenomena of lakes and reservoirs have been largely explained by the loading concept of nutrients such as nitrogen and phosphorus (OECD, 1982; Vollenweider, 1976) that are known as a key component controlling the trophic state. Thus, phosphorus (P) and nitrogen (N) are typically considered the major limiting nutrients regulating primary productivity of lentic and lotic systems. However, some studies have demonstrated that silica (or carbon) is also occasionally proposed as an important element in understanding lake nutrient cycling along with the nitrogen and phosphorus (Goldman, 1960; Schelske and Stoermer, 1972). However, little is known about the silica dynamics in Korean lentic systems.

The silica dynamics is important in understanding seasonality of algal biomass and composition because seasonal changes of silica concentration reflect the standing crop of diatoms, resulting in a contribution of total algal biomass. It is known that when thermal stratification is strong, it acts like a barrier to restrict the silica flux from the deep water. Numerous studies of phytoplankton seasonality in the North America and Europe showed that the collapses of spring diatom bloom occur in summer period as silica was rapidly depleted in the euphotic zone due to the isolation of nutrient supplies from deep region below the thermocline. Consequently, algal blooms during fall occur because of dramatic upsurges of silica from the bottom. In the mean time, the silica content of river waters tended to be homogeneous and showed little response to changes in discharge rates (Edwards and Liss,

1973). These observations demonstrated that temporal variations of silica are induced by the internal loading processes rather than the external inputs.

Conversely, White and Blum (1995) and Dickson (1975) explained that in-lake silica was mainly originated from the watershed and that silica concentrations had a positive functional relation with the magnitude of flood. Based on these studies, silica is controlled by a combined effect of internal processes and external loading. Recently Ha *et al.* (1998) showed a seasonal succession of diatoms in Nakdong River, Korea and pointed out an importance of rainfall pattern on the dynamics of silica and phytoplankton biomass along with water temperature. Still,

spatial and temporal dynamics of silica and its influence to algal biomass are not known in large man-made lakes. In this study, we demonstrate longitudinal and seasonal distribution of silica patterns in Taechung Reservoir, which is morphologically complex system. The objective of the study is to identify the horizontal and longitudinal patterns and seasonal variation of silica and to compare major factor influencing the silica concentration in the system.

Materials and Methods

Descriptions of the study site : Taechung Reservoir is the morphologically complex man made lake that has a surface area of $6.8 \times 10^7 \text{ m}^2$

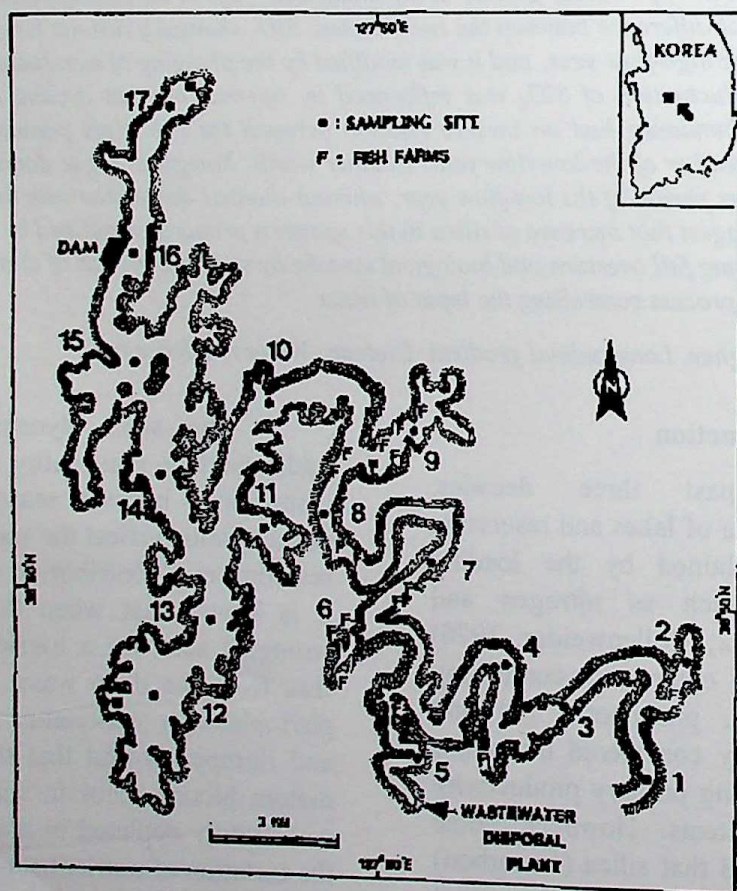


Fig. 1 : The map showing the sampling sites. The alphabet of "F" in the figure indicates the location of fish farm within the lake.

and a volume of $14.3 \times 10^8 \text{ m}^3$ with a mean depth of 21.2 m and maximum depth of 69 m at an elevation of 80 m MSL (Mean Sea Level). The selection of sampling sites in this system was

based on the morphometry along the longitudinal axis and the position of external nutrient loads to the system (Fig. 1). Along the main axis of the reservoir, 17 sampling sites were chosen for the

study. As shown in Fig. 1, nine mainstem sampling locations are sites 1, 3, 4, 7, 8, 10, 14, 15 and 16, and eight embayment sampling locations are sites 2, 5, 6, 9, 11, 12, 13, and 17. The distance from Site 1 to Site 17 is about 50 km.

Sample collection and analysis : Epilimnetic water samples were collected from these 17 sites twice each month during two years of the high-flow year (1993) and low-flow year (1994). The year of 1993 was most intense monsoon year since the construction of Taechung Reservoir and the year of 1994 was most drought year. The detailed hydrological contrasts between the two years are available in An and Jones (2000b). Water samples were covered to prevent exposure to direct sunlight, stored in ice, and either preserved or analyzed in the laboratory within 12 - 36 hours for the sample analyses. Silica concentrations in the surface water were

measured in triplicate using the molibdosilicate method (APHA, 1985). For identification of algal species, surface un-filtered samples were preserved in Lugol's solution (1 : 100), and a Sedgwick-Rafter cell counter under inverted microscope was used for algae counting (APHA, 1985). Hydrological conditions were considered an important component influencing the silica dynamics, so that theoretical water residence time (TWRT, days) was calculated after the approach of Knowlton and Jones (1990). Surface area of the reservoir, its volume and inflow on each sampling date, season and location in the reservoir were used to estimate the length of time that water had been in the reservoir prior to collection. To estimate the TWRT, the reservoir was considered completely homogeneous along its main axis so that the TWRT was based simply on the volume of water uptake from a particular location and the sum of

Table-1 : Spatial and interannual distribution of silica (SiO_2 , mg/L). Sampling sites of the mainstems and embayments are arranged by order of location along the axis of the reservoir from the headwaters (Site 1) to downlake (Site 17) and the values at each site in the high- and low-flow year are averages of 11 and 14 samples, respectively.

Sites	High-flow year		Low-flow year	
	Annual Mean	Max. - Min.	Annual Mean	Max.-Min.
Mainstems				
S-1	6.4	0.4 - 9.7	2.8	0.9-6.2
S-3	6.1	0.4 - 9.4	2.6	1.0 -6.1
S-4	6.2	1.0 - 8.0	2.6	1.0-6.0
S-7	5.1	0.8 - 8.3	3.2	1.0 -7.2
S-8	4.5	1.3 - 7.7	3.1	0.6 - 7.2
S-10	3.6	0.1 - 6.6	2.6	0.4 - 5.7
S-14	3.1	0.4 - 5.8	2.5	0.3-4.9
S-15	2.5	0.3 - 4.8	2.0	0.3 - 4.4
S-16	2.5	0.3 - 4.9	2.0	0.4 - 4.3
Embayments				
S-2	5.9	0.3 - 9.0	2.4	0.8-5.3
S-5	5.8	0.9-8.3	2.5	0.7-6.6
S-6	5.2	0.9 - 8.2	2.7	0.6-6.6
S-9	3.5	1.1 - 6.5	2.7	0.3 - 6.6
S-11	2.8	0.3-5.2	2.5	0.3-5.2
S-12	2.7	0.2-5.5	2.4	0.4-4.8
S-13	2.8	0.1 - 5.6	2.7	0.4 - 5.2
S-17	2.1	0.3 - 4.7	2.0	0.5- 4.3
Mainstem Avg.	4.4	0.3-9.7	2.6	0.3-7.2
Embayment Avg.	3.9	0.1-9.0	2.5	0.3-6.6

Table-2 : The paired sample tests of silica, based on the years and locations. The high-flow year and low-flow year were expressed as "H" and "L", respectively, the mainstem and embayment sites as a "ma" and "em", and the paired difference of the mean as a "D_m". The degree of the freedom (DF) was 7 for all comparison tests. In the both years, annual mean in the site 15 was same as that of site 16, so site 16 was deleted for the pairwise comparisons between the two locations.

Comparisons		D _m	t- value	p-value (2-tailed)
Flow	H _{ma} vs. L _{ma}	2.26	3.577	0.009**
	H _{em} vs. L _{em}	1.36	2.600	0.035*
	H _{ma} vs. H _{em}	0.84	4.189	0.004**
Site	L _{ma} vs. L _{em}	-0.03	-0.222	0.831
	H _{ma} vs. L _{em}	2.20	4.348	0.003**
Flow & site	L _{ma} vs. H _{em}	1.43	2.035	0.081

* $p < 0.05$; ** $p < 0.01$

inflows over the preceding months. Statistical tests were conducted using the analysis of SAS (SAS, 1991).

Results and Discussion

During the study, concentrations of silica (SiO₂) averaged 3.4 mg/L and ranged from 0.1 to 9.7 mg/L depending on the year and the location (Table 1). The world's average of silica is known as 13 mg/L for freshwater lakes (Wetzel, 1983), so the mean value during the two years was 74% lower than the world's average. Mean epilimnetic SiO₂ (4.2 mg/L) in the high-flow year was greater by about 1.6 fold relative to the low-flow year (2.6 mg/L; Table 1). The ranges of the minimum and maximum were greater in the headwater zones of mainstem and embayment sites than in the downlake zone, indicating that silica is greater in the riverine than in the lacustrine reach and that the longitudinal variation is greater in the riverine reach.

The paired sample test of SiO₂ was conducted using the analysis of SAS to demonstrate the effects of flow regime and sampling location on the silica variation. As shown in Table 2, in the mainstem sites SiO₂ was significantly ($t = 3.577$, $p < 0.01$) greater in the high-flow year than in the low-flow year. The effect of flow in the embayment sites was similar ($t = 2.600$, $p < 0.05$) to that of the mainstem sites. This outcome indicates that the high-flow resulted in greater silica in the epilimnion in both reaches

of the mainstem and embayment sites. In the mean time, the comparison of locations within the reservoir (mainstem vs. embayment sites) during the high-flow year showed that SiO₂ was significantly ($t = 3.577$, $p < 0.01$) greater in the mainstem sites than in the embayments. This greater silica in the mainstems seems to be a result of greater hydraulic loads in the mainstems than the embayments because in general, water renewal time in the embayment locations is shorter than that in the mainstems. During the low-flow year, there was no statistical difference ($t = -0.222$, $p = 0.831$) between the two reaches, implying that input of SiO₂ from the watershed was little. These hydraulic conditions resulted in weak horizontal variation of silica. The observation data demonstrate that the in-lake silica is mainly originated from the watershed and the magnitude of interannual and horizontal variations reflected the flow regime between the two contrast years. Such patterns of silica are similar to interannual and seasonal patterns of phosphorus as shown in Korean reservoirs (An and Jones, 2000b; An and Jones 2002).

Concentrations of SiO₂ declined from the headwaters to the dam in the high-flow year, but such pattern did not show in the low-flow year. According to regression analysis of SiO₂ based on three seasons average of the high-flow year, silica values were strongly correlated ($r = 0.98$; $n = 9$; $p < 0.001$) with the distance from the headwaters and declined linearly at the rate of 0.08 mg/L per km. However, when we plotted only the silica

Longitudinal and seasonal variations of epilimnetic silica.

data during the monsoon of the high-flow year, the

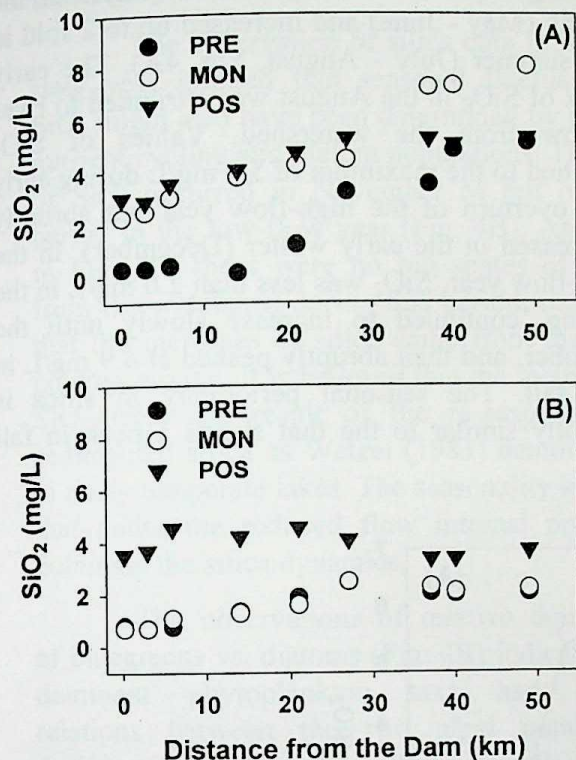


Fig. 2 : Seasonal variations of longitudinal SiO_2 during three seasons of premonsoon (PRE), monsoon (MON) and postmonsoon (POS) in the high-flow year (A, upper panel) and low-flow year (B, lower panel).

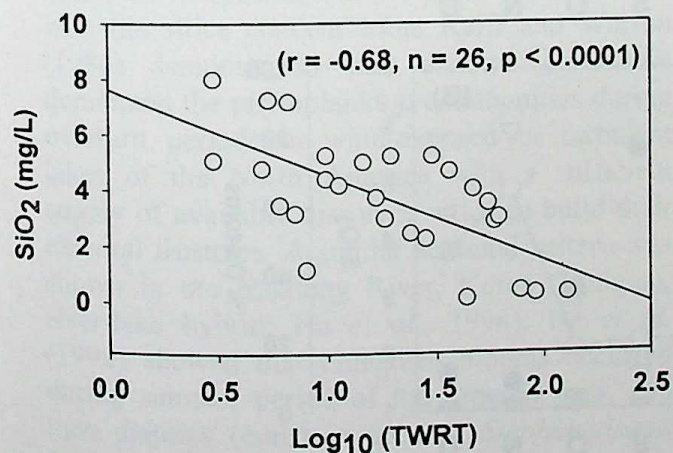


Fig. 3 : Relations between SiO_2 and log-transformed theoretical water residence time (TWRT) in the high-flow year. The value of "r" indicates a correlation coefficient.

regression slop increased by 0.12. Thus, there was a distinct longitudinal difference of silica between the premonsoon and monsoon season (Fig. 2A). Silica values during the postmonsoon showed a distinct decrease, especially in the headwater zone, compared to the monsoon and the downlake decline was evident (Fig. 2A). The distinct downlake decline in the high-flow year was a result of progressive decreases in external silica loads carried by floodwater during the summer monsoon. The positive relationship between silica and floods is consistent with previous findings from non-monsoon lakes in temperate regions (Dickson 1975; White and Blum 1995). As shown in Fig. 3, this supposition is supported by the inverse correlation ($r = -0.68$; $n = 26$, $p < 0.001$) between silica and theoretical water residence time (by site) during the high-flow year. In other words, increases of silica were most pronounced during the rapid flushing period. However, the relations were not evident in the low-flow year when mean TWRT was more than 100 days, and the pattern of longitudinal decline was not significant ($p = 0.10$, $n = 9$) due to the reduced flow.

The longitudinal pattern in silica during summer monsoon (July-August) of the high-flow year, however, seems to be modified by a density current. As shown in Fig. 3, there was a large difference in the longitudinal distribution of epilimnetic silica between the intense monsoon period of the high-flow year and the weak monsoon period of the low-flow year. As shown in Fig. 2, a sharp longitudinal decline of $> 40\%$ in SiO_2 occurred between location 27 and 37 km during the intense monsoon of the high-flow year, whereas during the weak monsoon of the low-flow year the difference between the two locations was little (Fig. 2B). We believe that the difference was due to a modification of the water movement. Based on vertical temperature profiles measured by An (2001), density current of interflow occurred between the two locations was evident, during the intense monsoon of the high-flow year. The study showed that interflow water in the metalimnion was more than $2 - 3^\circ\text{C}$ colder relative to the reservoir surface temperature. The

distinct discontinuity in the plunge point are shown in conductivity, measured by An and Jones (2000a).

A similar comparable interflow was observed in Lake Soyang during summer period when turbid inorganic solids dominated. These observations suggest that this decline in silica was not due to abiogenic sedimentation of suspended solids in the epilimnetic water (Wetzel, 1983) but was a result of hydrological modification of the interflow (An and Jones, 2000a). In the mean time, overall mean of silica was largest during the postmonsoon in the low-flow year, and the longitudinal differences were little during three seasons (Fig. 2B).

Concentrations of SiO_2 , based on mean of all sites, showed large seasonal fluctuations. In the high-flow year, SiO_2 was 2.4 mg/L in the spring (May - June) and increased up to 2 fold in the summer (July - August, Fig. 4A). The early peak of SiO_2 in the August was attributed to river inflow from the watershed. Values of SiO_2 reached to the maximum of 5.9 mg/L during early fall overturn of the high-flow year but abruptly decreased in the early winter (December). In the low-flow year, SiO_2 was less than 2.0 mg/L in the spring, continued to increase slowly until the October, and then abruptly peaked at 4.9 mg/L in the fall. The seasonal periodicity in silica is slightly similar to the that shows a peak in fall and

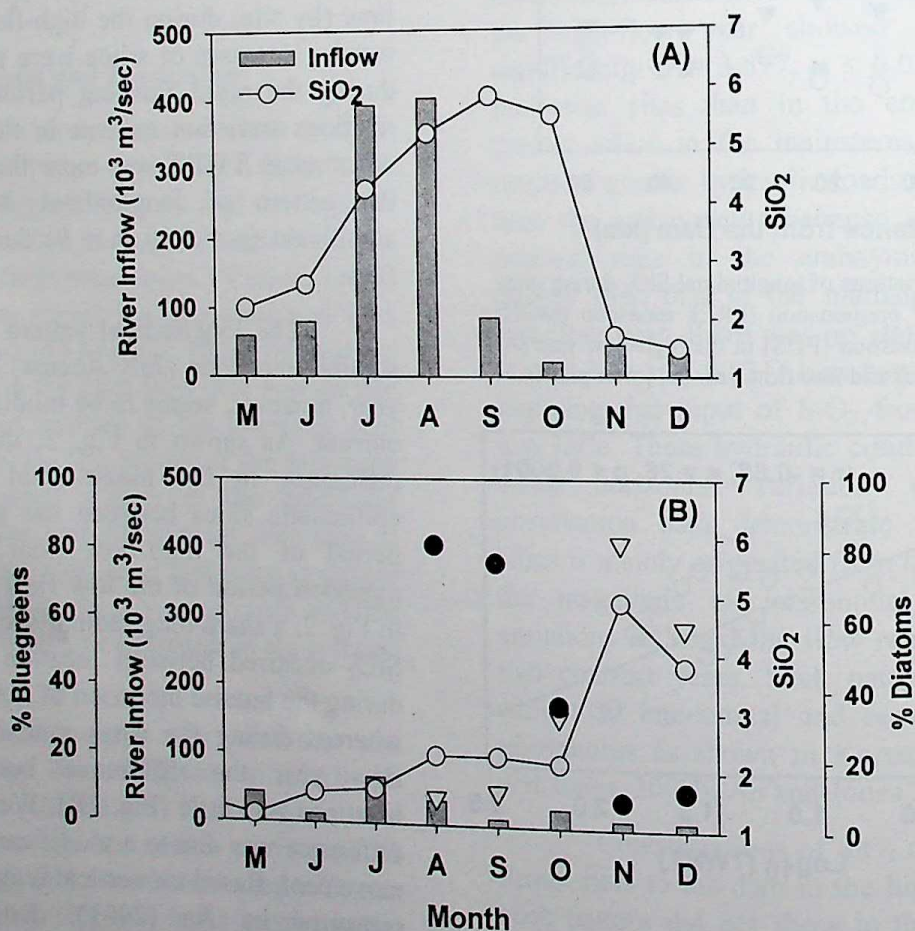


Fig. 4 : Monthly changes of SiO_2 and river inflow in the high-flow year (A) and low-flow year (B) and their relations to the relative dominance and bluegreen populations. In the Fig. 4B, dark circles and triangles indicate percent bluegreens and diatoms, respectively, which were estimated from each proportion of total cells.

low in winter and early spring (Lund 1950; Jorgensen 1957; Dickson 1975; Wetzel 1983).

The distinct difference, however, occurred during the intense monsoon of the high-flow year

between this system and lakes of the other regions, indicating that an alteration of the seasonality during the intense monsoon period.

The observation of silica data in the low-flow year implied that seasonal fluctuation of SiO_2 might also have been determined by internal nutrient cycling and diatom populations. The peak of SiO_2 occurred in November of fall overturn period in the low-flow year (Fig. 4B). As shown in Fig. 4B, there were no big spates of inflow from the watershed during this period, indicating that the increases of silica came from the water column mixing. It is believed that the increases of SiO_2 were the result of the re-suspension of sedimented silica as Wetzel (1983) demonstrated in many temperate lakes. The seasonality suggests that under the reduced flow internal processes dominate the silica dynamics.

The observations of relative dominance of bluegreens vs. diatoms (Fig. 4B) indicated that dominant phytoplankton taxa had inverse relations between the two algal populations during August-December of the low-flow year. The relative abundance of bluegreens declined rapidly from 8% in the August to 11% in the December, whereas diatoms increased up to 7 fold over the period and the peak was coincided with the silica concentration. Kalff and Watson (1986) demonstrated that diatoms commonly dominated the phytoplankton communities during overturn periods in wind-exposed or turbulent lakes of the North America with a sufficient supply of available dissolved silica to build their external frustules. A similar seasonal pattern was shown in the Nakdong River, Korea (Mulgum, river-lake hybrid; Ha *et al.*, 1998). Ha *et al.* (1998) showed that bluegreen blooms occurred during summer period of the drought year, and then diatoms (common species, *Stephanodiscus hantzschii*) dominated from late fall to next spring (mean cell density, 7.5×10^4 cells/ L). These findings indicate that the increased silica of Taechung Reservoir was due to the internal loading. During the period of the silica peak, *Melosira*, *Synedra*, and *Cyclotella* were the major taxa of the phytoplankton communities, indicating

that the internal loading of silica resulted in the dominance of diatoms. Whereas, in this period *Microcystis* and *Anabaena* were minor taxa and the proportion was less than 20% of the total (Fig. 4b). In other words, bluegreen algae dominated at the low SiO_2 (< 2.5 mg/L) during the summer period of the low-flow year, whereas diatoms dominated with the increase of SiO_2 in fall. This finding might support the hypothesis that availability of SiO_2 in lake water is involved with a shift of algal composition from bluegreen algae to diatoms and environmental attributes (Kilham, 1971; Sommer, 1985; Sterner, 1989; White and Blum, 1995). In addition, Reynolds *et al.* (1993) pointed out that the magnitude of the disturbance driven by rainfall or river inflow determines the phytoplankton seasonality and the biomass is closely associated with silica supplied from the watershed or internal loading. This hypothesis seems to be fit to this system, but we believe that many other factors such as phosphorus, nitrogen or light availability may be associated with the seasonality (An and Jones, 2000b; An and Park, 2002; An and Jones, 2002). The present study suggests that major in-reservoir silica was supplied from the watershed during flow-dominated summer season, so the consistent longitudinal declines in the silica concentrations were evident, although there was a spatial modification of the concentration in the plunge point in the mid-reach. However, internal loading of silica during fall overturn and the biological up-take by seasonal growth of diatom communities were also considered as an important process supplying the silica to the epilimnion from hypolimnion during the drought period when the flow from the watershed was base condition. Based on the preliminary results, silica may be reduced during strong thermal stratification in the lacustrine reach of the reservoir. These conditions would accelerate diatom blooms in the reservoir through the increase of silica during the fall overturn. This outcome indicates that silica is also important plant nutrient influencing the algal blooms to the reservoir as well as nitrogen and phosphorus.

Further studies should be done for efficient managements of the reservoir.

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Evaluation of growth potential of Crimean juniper (*Juniperus excelsa* Bieb.) seedlings for the first growing season under Tekir forest nursery conditions in Kahramanmaras, Turkey.

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Abstract : In this study, growth potential of Crimean juniper (*Juniperus excelsa* Bieb.) seedlings for the first growing season under Tekir Forest Nursery conditions in Kahramanmaras was evaluated. The height growth of Crimean juniper seedlings was relatively close to that of Lebanon cedar (*Cedrus libani* A. Rich.) seedlings produced in the same nursery, but their root collar diameters were fairly lower than that of Lebanon cedar seedlings. According to coniferous seedling standards of Turkish Standards Institute, the height growth of Crimean juniper seedlings was fairly good, but their root collar diameters were slightly small. In this respect, that 2+0 or 1+1 Crimean juniper seedlings are used in reforestation activities in the region would be more useful than 1+0 seedlings.

Key words : *Juniperus excelsa*, Seedling, Growth, Reforestation.

Introduction

Crimean juniper (*Juniperus excelsa* Bieb.) is one of the major tree species of Turkey. In Turkey, it generally occurs in northern, western, central, and southern Anatolia, especially the Taurus and Anti-Taurus Mountains (Yaltirik, 1993) and is generally distributed at elevations between 500 and 2500 meters on the areas going up to the boundaries of steppe in the interior parts of the mountains, and resistant to drought and frost damages (Saatcioglu, 1969). Crimean juniper forms pure and mixed stands in Turkey and usually occurs on shallow, stony, and poor sites. Therefore, this species is very important for reforestation activities aiming at the prevention of soil erosion especially in arid sites.

However, juniper seeds have embryo dormancy and sometimes both embryo and seed coat dormancy (Saatcioglu, 1971) and germination of juniper seeds is often delayed (Young and Young, 1994). Therefore, seeds of Crimean juniper usually germinate in the second spring after sowing (Elicin, 1977; Demirci and Avsar, 2000). Likewise, germination percentage of the seeds has also been low due to probably low filled seed proportions. In this respect, Crimean juniper seedling production is very

limited in the forest nurseries, and there is very limited usage of Crimean juniper seedlings for reforestation activities.

Lebanon cedar (*Cedrus libani* A. Rich.) and black pine (*Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe) are the species largely used for reforestation activities in Turkey, and seedlings of these species have been generally used as 1+0 and 2+0, respectively in the Kahramanmaras region. According to Saatcioglu (1969), Lebanon cedar, black pine, and junipers are known as fast, moderately fast, and slow growing species, respectively. Khatkhat and Sheikh (1981) and Ciesla *et al.* (1998) stated that the growth of Crimean juniper trees is very slow and slow, respectively. Likewise, Eler (1988) determined that Crimean juniper grew slower in terms of diameter and height growth than Lebanon cedar and black pine. However, in Turkey, there are not sufficient studies regarding the growth of Crimean juniper seedlings under the nursery conditions having more suitable site conditions than that of its natural sites.

In this study, growth potential of Crimean juniper seedlings for the first growing season under Tekir Forest Nursery conditions was evaluated. For this, root collar diameter and

height values of 1+0 Lebanon cedar, black pine, and Crimean juniper seedlings grown at Tekir Forest Nursery in Kahramanmaras were compared. Thus, especially the possibilities of using 1+0 Crimean juniper seedlings in reforestation activities in the region were discussed.

Materials and Methods

The study was carried out at Tekir Forest Nursery (37°53'N, 36°37'E). The nursery is located in Tekir town of Kahramanmaras province and at an elevation of 980 meters. In the nursery, soil texture is generally sandy clay loam, and soil reaction (pH) values range 7.86 to 8.20 (Kimyon *et al.*, 1994). The mean annual temperature and rainfall of Tekir are 11.2°C and 400.9 mm, respectively. These climatic data were obtained by adjusting the data of Goksun Meteorologic Station (Anonymous, 1974) that is the nearest station to Tekir.

Measurements of the seedlings were carried out on 18 October 2000 by observing bud formation of the seedlings at the end of the growing season. For each tree species, 60 seedlings were randomly chosen from the seedbeds. Root collar diameter measurements were taken just above soil surface and height measurements were taken between soil surface and the top of the terminal bud. Root collar diameters and heights were measured to the nearest 0.01 and 1 mm, respectively. Seed sources and sowing dates of the species measured in the nursery are given in Table 1.

As indicated in the table, Crimean juniper and Lebanon cedar seeds were sown in the winter and seeds of black pine were sown in the spring. Crimean juniper seeds germinated in the second spring after sowing, while seeds of the other two species germinated in the first spring. Therefore, at the time of the measurement, the seedlings of three species were 1-year-old. Besides, Lebanon cedar and black pine seedbed densities were similar, but Crimean juniper seedbed density was lower than that of the other two species due to

low germination percentage of Crimean juniper seeds.

After obtaining root collar diameter and height data of 1+0 seedlings, statistical values regarding these data were found. Besides, in order to determine whether there is a statistically significant difference in root collar diameter and height values of the species, one-way analysis of variance (ANOVA) was performed. Then, Duncan's multiple range test was performed to determine different groups from each other (Kalipsiz, 1981). Statistical analysis of the data was carried out by using Statgraphics 5.0 program. In addition, the distributions of root collar diameter and height values of the species to coniferous seedling standards of Turkish Standards Institute (Anonymous, 1988) were examined.

Results

Root collar diameter : Statistical values regarding the root collar diameter values of the species are given in Table 2. As seen in the table, the mean root collar diameters were 2.65, 1.90, and 1.60 mm for Lebanon cedar, Crimean juniper, and black pine seedlings, respectively.

According to the results of one-way analysis of variance (Table 2), the root collar diameter values of three species were significantly different ($p=0.001$). According to the results of Duncan's multiple range test (Table 2), there was not any homogeneity ($p=0.05$) in terms of the root collar diameter values among three species. In other words, the highest root collar diameter growth was in Lebanon cedar, then Crimean juniper and black pine. Root collar diameters of Lebanon cedar seedlings were fairly larger than of Crimean juniper and black pine seedlings.

On the other hand, according to coniferous seedling standards of Turkish Standards Institute (Anonymous, 1988), in terms of the root collar diameter values, 95.0% and 5.0% of Lebanon cedar seedlings were good-quality and poor-quality seedlings, respectively.

These proportions were 41.67% and 58.33% for Crimean juniper seedlings, and 5.0% and 95.0% for black pine seedlings, respectively.

Height : Statistical values regarding the height values of the species are given in Table 3. As seen

Table – 1 : Seed sources and sowing dates of the species measured at Tekir Forest Nursery.

Tree species	Seed source	Sowing date
Crimean juniper	Heyiktepe (Kahramanmaras)	22 December 1998
Lebanon cedar	Heyiktepe (Kahramanmaras)	25 December 1999
Black pine	Camurlu (Goksun)	06 May 2000

Table – 2 : Statistical values and the results of the one-way ANOVA and Duncan's multiple range test regarding the root collar diameter values of the species.

Tree species	Mean* (mm)	SD (mm)	Min. (mm)	Max. (mm)	CV (%)	F-ratio	P-value
Lebanon cedar	2.65a	0.53	1.67	4.26	20.15	107.089	0.0000
Crimean juniper	1.90b	0.39	0.95	2.90	20.30		
Black pine	1.60c	0.24	1.11	2.19	14.79		

SD : Standard deviation, CV : Coefficient of variation

*Means followed by the different letters are significantly different ($p=0.05$)

Table – 3 : Statistical values and the results of the one-way ANOVA and Duncan's multiple range test regarding the height values of the species.

Tree Species	Mean* (cm)	SD (cm)	Min. (cm)	Max. (cm)	CV (%)	F-ratio	P-value
Lebanon cedar	10.75a	2.48	4.50	17.40	23.06	223.805	0.0000
Crimean juniper	9.76b	2.21	4.10	17.30	22.61		
Black pine	3.65c	0.94	2.00	6.40	25.81		

SD : Standard deviation, CV : Coefficient of variation

*Means followed by the different letters are significantly different ($p=0.05$).

in the Table, the mean heights were 10.75, 9.76, and 3.65 cm for Lebanon cedar, Crimean juniper, and black pine seedlings, respectively.

According to the results of one-way analysis of variance (Table 3), the height values of three species were significantly different ($p=0.001$). According to the results of Duncan's multiple range test (Table 3), there was not any homogeneity ($p=0.05$) in terms of the height values among three species. In other words, the highest height growth was in Lebanon cedar, then Crimean juniper and black pine. However, the height values of Lebanon cedar and Crimean juniper seedlings were relatively close to each

other, but the height values of black pine were different.

On the other hand, according to coniferous seedling standards of Turkish Standards Institute (Anonymous, 1988), in terms of the height values, 90.0%, 8.33%, and 1.67% of Lebanon cedar seedlings were the best-quality, good-quality, and poor-quality seedlings, respectively. These proportions were 3.33%, 5.0%, and 91.67% for black pine seedlings, respectively. 100.0% of Crimean juniper seedlings were the best-quality seedlings.

Discussion

In a study carried out on 1-year-old, 60 Crimean juniper seedlings on 17 December 1996 at Tekir Forest Nursery, the mean height was found to be 13.1 cm (Demirci and Avsar, 2000). In another study carried out on 1-year-old, 30 seedlings for each tree species in September and October 1999 in the same nursery, it was reported that the mean heights were 12.0, 11.8, and 6.2 cm for Crimean juniper, Lebanon cedar, and black pine, respectively (Akbaba, 2000). These results also indicate that Crimean juniper seedlings have a height growth potential close to that of Lebanon cedar seedlings for the first growing season under Tekir Forest Nursery conditions.

In fact, Gokmen (1970) stated that junipers grow fast for early years after establishment, and then their growth decreases. Eler and Cetin (1994) also stated that Crimean juniper seedlings grew fairly well under the nursery conditions. Eler and Karakus (1994) observed that Crimean juniper seedlings grown under the nursery conditions had better height growth than the natural seedlings of which growth was very slow. This most probably results from being more suitable of the nursery site conditions such as water, nutrient, and soil depth, etc.

On the other hand, considering the height/root collar diameter (mm/mm) ratio, this ratio was found to be 51.37, 40.57, and 22.81 for Crimean juniper, Lebanon cedar, and black pine, respectively. Although Crimean juniper seedlings had lower seedbed density than that of the other two species, it was observed that Crimean juniper seedlings could not produce large diameter seedlings. In fact, the studies regarding *Picea orientalis* (Eyuboglu *et al.*, 1984) and *Pinus brutia* (Keskin, 1992) revealed that low seedbed density affected root collar diameter positively, while seedbed density did not affect seedling height.

According to coniferous seedling standards of Turkish Standards Institute (Anonymous, 1988), in terms of height growth, all of the 1+0 Crimean juniper seedlings measured in the study are the best-quality seedlings. It has been suggested that the root

collar diameters of coniferous seedlings to be used should be at least 2 mm (Anonymous, 1988). 58.33% of Crimean juniper seedlings have the root collar diameters that are lower than this value. Therefore, even height growth of 1+0 Crimean juniper seedlings grown at Tekir Forest Nursery is good; it is possible to say that the root collar diameters of these seedlings are insufficient for reforestation activities. Likewise, Khattak and Sheikh (1981) reported that although 1-year-old Crimean juniper seedlings can be outplanted, 2-year-old stock is sturdier and better able to stand the rigours of field planting. Urgenc (1986) also emphasized that small diameter seedlings are more sensitive to high temperatures on the soil surface than large diameter seedlings. Therefore, that 2+0 or 1+1 Crimean juniper seedlings are used in reforestation activities in the region would be more useful than 1+0 seedlings. However, these evaluations should also be supported by field tests.

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Certain haematological responses in swiss albino mice following exposure to textile dye wastewater.

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Abstract : Adults Swiss mice were administered 5% solution of textile industry wastewater orally for 25 days and haematological parameters like RBC, WBC, Hb, and PCV were studied. Red cell indices like MCV, MCH and MCHC were calculated. Results indicate significant reduction in RBC, Hb and PCV levels. It is inferred that toxic effluents cause metabolic alteration in erythrocytes and reduce their Hb carrying capacity.

Key words : Textile dye wastewater, Haematological parameters, Swiss albino mice.

Introduction

Chemicals are integral part of every day life at Sanganer, an industrial town near Jaipur where the textile printing industries are located. Directly or indirectly, these chemicals greatly affect the life span of people residing there. In recent years, the contamination of the environment at Sanganer by toxic textile dye wastewater is increasing. The workers are exposed to the textile effluents with no control over the amount or frequency of exposure at work. In Sanganer, almost 400 textile units were reported which discharge approximately 4000 kl of dye wastewaters everyday in adjoining shallow pools and the drain. These textile wastewaters are highly toxic in nature as they contain large varieties of dyes such as azoic, indigo, and aniline used in various processes. The wastewaters also contain bleaching agents, salts, acids/alkalies, heavy metals etc. Analysis of the dye wastewater showed presence of heavy metals such as Cd, Cu, Zn, Cr and Pb in higher concentration more than the permissible limits prescribed by WHO. The textile wastewaters are either highly alkaline or highly acidic and have high BOD, COD, suspended solids, etc. (Sharma *et al.*, 1999). This polluted water adversely affects the whole ecosystem of Sanganer. The adverse effects such as fall in erythrocyte count and Hb content have been reported in fishes following exposure to lead and other pollutants (Hymavathi & Rao, 2000; Hardikar and Gokhle 2000). Kurde and Singh

(1995) also found textile water more toxic with decreased RBC count and reduced Hb content in Wistar rats. However, the reports on mammals, especially on haematology, are scanty. Hence, this work was undertaken to study the toxic effects of textile dye wastewater on haematological parameters in adult mice. It is hoped that the study on blood would find extensive application in medical practice. It is expected that the parameters chosen are of clinical interest and will help in knowing about the deleterious effects of textile dye wastewater on animals and human health.

Materials and Methods

Healthy adult Swiss albino mice weighing 25-30 grams were selected for the study. They were maintained on standard pellet diet (Manufactured by Hindustan Lever Limited, India) and water *ad libitum*. The animals were equally divided into two groups of 5 mice each. The 1st group served as control and was fed a normal pellet diet. The 2nd group was given 5% solution of textile waste effluent water *ad libitum* and standard diet for consecutive 25 days. After finding the LD₅₀ value by applying Finney's (1971) method, the concentration of the experimental solution was decided. Duration for the experiment was decided keeping in view the physical discomfort of the treated mice during experimental work. The animals were autopsied for various haematological studies after 25 days of exposure. Total RBC, WBC count (Dacie and

Lewis, 1977), Hb concentration (standard Sahil's method), PCV, MCV, MCH, and MCHC (Wintrobe *et al.*, 1976) were determined. The data obtained were statistically analysed using student 't' test.

Results and Discussion

The results of textile wastewater treatment on haematological parameters are shown in Table 1. The data indicate a profound change in haematological profile of adult mice treated with 5% concentration of textile dye wastewater for 25 days. RBC counts declined significantly (-52.50%) as compared to the control value. The decline in WBC was -32.5% when the experimental value was 2700 / cu mm. These changes were accompanied by a fall of -34.80% and -48.20% in Hb and PCV respectively. There was a decrease (-17.70%) in MCV while MCH and MCHC increased +68.05% and +7.67% respectively in effluent exposed mice as compared to control.

The haematological results clearly indicate the response of the organism to changing environmental conditions. The RBC count and Hb concentration declined after treatment with textile effluents. The reduction in these values in mice may be due to the bone marrow depression caused by drinking dye wastewater (Case *et al.*, 1979; Mitruka and Rawnsky, 1977; Gibson and Goldberg, 1970). Interaction of toxic substances in the wastewater with red blood cells may cause metabolic alteration in erythrocyte and Hb carrying capacity of the blood, consequently resulting in lower Hb count (Oda *et al.*, 1980). Similar observations have been reported due to the lead poisoning in human beings (Berk *et al.*, 1970). Further, it has been observed that these toxic dyes have a destructive action on the erythrocytes, thus reducing their survival time by increasing their fragility (Shukla *et al.*, 2000). Our findings are in agreement with the observation reported by Chandravathy *et al.* (1996) in mice with lead nitrate.

Table - 1 : Haematological changes following exposure to textile dye wastewater effluent in swiss albino mice.

Parameters	Control	Experimental	% Variation
RBC ($\times 10^6$ / mm ³)	8.78 ± 1.46	$4.17 \pm 1.61^*$	-52.50 %
WBC (per cu mm.)	4000 ± 3.39	$2700 \pm 2.54^*$	-32.50 %
Hb (gm %)	13.66 ± 2.45	$8.90 \pm 1.15^{**}$	-34.80 %
PCV (%)	40.60 ± 2.60	$21.00 \pm 2.60^*$	-48.20 %
MCV (fl)	47.79 ± 11.46	39.33 ± 9.96	-17.70 %
MCH (pg)	15.56 ± 6.73	26.15 ± 17.59	68.05 %
MCHC (%)	33.75 ± 5.99	36.34 ± 9.61	7.67 %

*Significant at 1% probability

**Significant at 5% probability

Kurde and Singh (1995) have reported in male Wistar rats significant decrease in RBC count with textile effluents. Similar observations regarding decrease in RBC and Hb have been reported in rats by the inhalation of toxic smoke by Lal *et al.* (1993). The decrease in Hb concentration after exposure for 25 days to textile effluents may be the result of increase in oxidative damage of red cells due to ribosomal abnormality, which is caused by toxic action of heavy metals present in textile dye wastewater (Albahary, 1972). The degree of anemia is directly related to the concentration and exposure time to toxics.

Kurde and Singh (1995) also found textile water more toxic with reduced Hb content in Wistar rats.

In this investigation white blood cell count also declined. This decline may be due to toxic effect of water on haemopoietic system causing reduction in WBC count. However, Chandravathy *et al.* (1996) reported no significant change in leucocyte count with lead nitrate in mice. The insignificant decrease in WBC count resulted in low weakening of the animals and finally loss in body weight.

In the present study, the packed cell volume decreased considerably after exposure to textile waste effluent, which is accompanied with similar fall in RBC count because of toxic damages. Fall in the value of PCV may be correlated with the degree of anemia due to toxic action of wastewater in mice. The PCV values are important in determining the effect of stresses on the health of the animal and are indicator of oxygen carrying capacity of the blood (Larsson *et al.*, 1985). The low PCV would indicate anemia (Wepener and Vuren, 1992). Kurde and Singh (1995) reported textile effluents to be more toxic and fall in PCV in male Wistar rats. Similar conclusions have been reported after administering lead nitrate to swiss albino mice (Chandravathy *et al.*, 1996) and after toxic gas exposure to human beings (Posin *et al.*, 1978). The anemia depends on the decrease in red cell count, PCV value and haemoglobin concentration. Decrease in PCV values may be related to decreased RBC production.

In the present study red cell indicators like MCV, MCH and MCHC are dependent on the RBC count, Hb concentration and PCV value. In this experiment, a fall in MCV has been reported, which may be due to fall in red cell count and PCV value. The rise in MCH and MCHC indicate the toxic effect of wastewater. This insignificant change can be explained due to defect in haemopoietic system in response to toxic action of wastewater. The slight increase in MCH and MCHC values indicate a reciprocal relationship with RBC, Hb and PCV. Work of Chandravathy *et al.* (1996) also indicate reciprocal relationship of red cell indices MCH and MCHC with RBC, Hb and PCV after treatment with lead nitrate in swiss albino mice. The values of RBC, Hb, PCV, and MCV indicate decrease, which may be due to inhibition of Hb synthesis and aerobic glycolysis. The inhibitory effect of the trace elements on the enzyme system in the synthesis of Hb may be the cause of this decline.

From the present study it is clear that the dyes and the heavy metals present in the textile wastewater inhibit Hb synthesis and have a

deleterious effect on the haemopoietic system and blood cell counts.

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Seasonal variations in compostability and production of vermiprotein by *Eisenia fetida*.

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Abstract : The potential of *E. fetida* to degrade wastes into vermicompost and to produce vermiprotein in the form of worm-biomass during different seasons was evaluated. Results revealed that the environmental factors prevailing during different seasons did influence directly the life activities of the worm and indirectly the compostability of the wastes. Feeding activities of *E. fetida* reduced the time of production of an efficient organic pool with energy reserves as vermicompost. Further, the amount of vermicompost produced by the worm activity depended primarily on the environmental factors and secondarily on the nature of organic wastes.

Key words : Seasonal environmental factors, Vermicompost, Vermiprotein, Epigeic earthworm, *Eisenia fetida*.

Introduction

Epigeic earthworms have the potential to convert organic wastes into valuable vermicompost for plant growth and vermiprotein for use as animal feed in poultry and aquaculture. Fosgate and Babb (1972) suggested utility of epigeic earthworms in composting procedures. Edwards (1998) pointed out that several earthworm species from tropical and temperate regions have ability to breakdown organic wastes. Reinecke *et al.* (1992) recommended the use of earthworms like *Eudrilus eugeniae*, *Eisenia fetida* and *Perionyx excavatus* in laboratory and field composting procedures. *E. eugeniae* was preferred for processing agricultural organic wastes while *E. fetida* for kitchen and urban waste at community level and in house holds (Kale, 1998). Much attention was given to *E. eugeniae*, as it is voracious feeder and prolific breeder. Due consideration has also been given to *E. fetida* in composting organic wastes in laboratory as well as field conditions and the recovery of vermiprotein and vermicompost.

Literature revealed that there was paucity of work dealing with the influence of epigeic earthworms on compostability of wastes from agriculture and agro-based industries and the influence of these wastes on growth and

reproduction of the worms. *Eisenia fetida* being a temperate peregrine earthworm has well adapted to climatic conditions of both temperate and tropics. Northeast region of Karnataka is known for cultivation of variety of food crops such as redgram, blackgram, greengram, jowar, wheat, rice, bengalgram, millets and oil seeds. Wastes generated in agricultural fields and agro-based industries are not properly recycled for nutrient recovery. Hence, the present work was undertaken to find out the potential of this worm to degrade these wastes and convert into vermicompost and vermiprotein during different seasons.

Materials and Methods

Collection of organic wastes : Dried organic wastes generated at agricultural fields during harvesting were collected in quantity enough for experimental work. These wastes were from *Cajanus cajana* [Redgram pod husk (Rdph)], *Phaseolus mungo* [Blackgram pod husk (Blph)], *Phaseolus radiatus* [Greengram pod husk (Ggph)], *Triticum aestivum* [Wheat straw (Whst)], *Oryza sativa* [Rice straw (Riss)], *Sorghum ulgare* [Jowar straw (Jwrs)], *Dicanthium annulatum* [Grass straw (Grss)], *Parthenium hysteroporus* [Parthenium waste (Prtw)], *Cicer arietinum* [Bengalgram grain husk

(Bngh) and mixed organic waste (Mixw). For convenience, these wastes were grouped, on the basis of their physical structure, into grain based (Bngh), straw based (Riss, Whts, Jwrs), garden wastes (Grss, Mixw), pod-based wastes (Rdph, Ggph, Blph) and weed waste (Prtw). Later, they were chopped into small pieces (0.5"– 1.0") and stored for future studies. Simultaneously, sufficient quantity of urine-free cattle manure was brought from cattle shed, sun dried and powdered to get 0.2 mm particle size.

Preparation of culture beds and inoculation of worms : The powdered cattle manure was amended to individual organic wastes in the ratio of 1 : 5 (v/v) for maintaining proper (25-30 : 1) C : N ratio. These were sprayed with tap water in order to impart moisture content of about 75-80% and kept for one week for thermal stabilization, initiation of microbial degradation and softening of wastes for easy ingestion by the worms. Culture beds in triplicates of each diet for 30, 60 and 90 days intervals were prepared at the onset on each season by transferring individual amended diet to round plastic container (481 cm³ volume with 53 cm² surface area) with pin-holed lid for ventilation and prevention of predators. Five sexually matured (5-6 week aged) worms were isolated from stock culture and after noting weight they were inoculated to each culture container in the ratio of 1 : 50 w/w (worm and waste diet). Simultaneously, to know the role of earthworm in composting organic wastes, another set of triplicate containers of each amended diet, but without worms, served as controls. Experiments were set up with the onset of each [summer (February-May), monsoon (June-September) and winter (October-January)] season. Prevailing seasonal environmental conditions (temperature and RH) during experiments were 25.0-33.82°C (\bar{X} 30.49°C) and 34.57-55.64% (\bar{X} 42.63%), 26.14-29.67°C (\bar{X} 28.45°C) and 53.78-81.71% (\bar{X} 67.01%) and 24.39-30.21°C (\bar{X} 26.50°C) and 56.71-82.07% (\bar{X} 67.46%) in summer, monsoon and winter seasons respectively. Culture containers were maintained in uncontrolled room conditions with

daily sprinkling required amount of tap water to impart about 75-80% moisture.

To determine the compostability of organic wastes by *E. fetida* in relation to time, triplicates of each amended diet were terminated at the end of 30, 60 and 90 days intervals during each season.

Control sets were terminated only at the end of 90 days along with experimental sets of diets, since much of the organic waste in those of 30 and 60 days intervals remained unchanged. At the termination of each experiment, observations were made in respect of number of old and new clitellate worms, sub-clitellates, juveniles, cocoons and their weight to determine the worm biomass. Gross biomass was calculated by adding the weight of all new individual stages to the final weight gained by inoculated worms during the 30 / 60 / 90 days. Percent vermicompost produced from each diet was calculated by isolating degraded material with the help of 0.2 mm sieve. Statistical significance of data of the correlation coefficient was obtained by Pearson's one tailed test.

Results and Discussion

The environmental factors during different seasons had direct influence on life activities of the worm and compostability of wastes. This indirectly affected the production of vermicompost and vermicoprotein.

Vermicompost : The quantity of vermicompost produced from all diets during winter (favorable) season was more than that during monsoon followed by summer season (Fig. 1). Amount of vermicompost produced in different seasons significantly correlated with the total increased number and biomass of the worms over the time (30 - 90 days). The range of percent vermicompost production in different diets during winter, monsoon and summer seasons were 31.60 (Whts) and 50.32 (Grss), 28.06 (Whts) and 45.80 (Grss) and 26.95 (Whts) and 38.43 (Mixw) at 30th day; 51.26 (Prtw) and 63.72 (Grss), 48.92 (Whts) and 61.00 (Bngh) and 36.79 (Blph) and 56.00 (Bngh) at 60th day and 54.86 (Prtw) and 73.82

Compostability and production of vermiprotein by E. fetida.

(Bngh), 55.42 (Whts) and 66.76 (Mixw) and 41.12 (Whts) and 61.60 (Mixw) at 90th day. The percent compost produced at 90th day during winter, monsoon and summer seasons ranged

between 31.22 (Rdph) and 53.18 (Grss), 32.86 (Rdph) and 57.14 (Grss), and 28.72 (Blph) and 40.86 (Riss) respectively (Fig. 1).

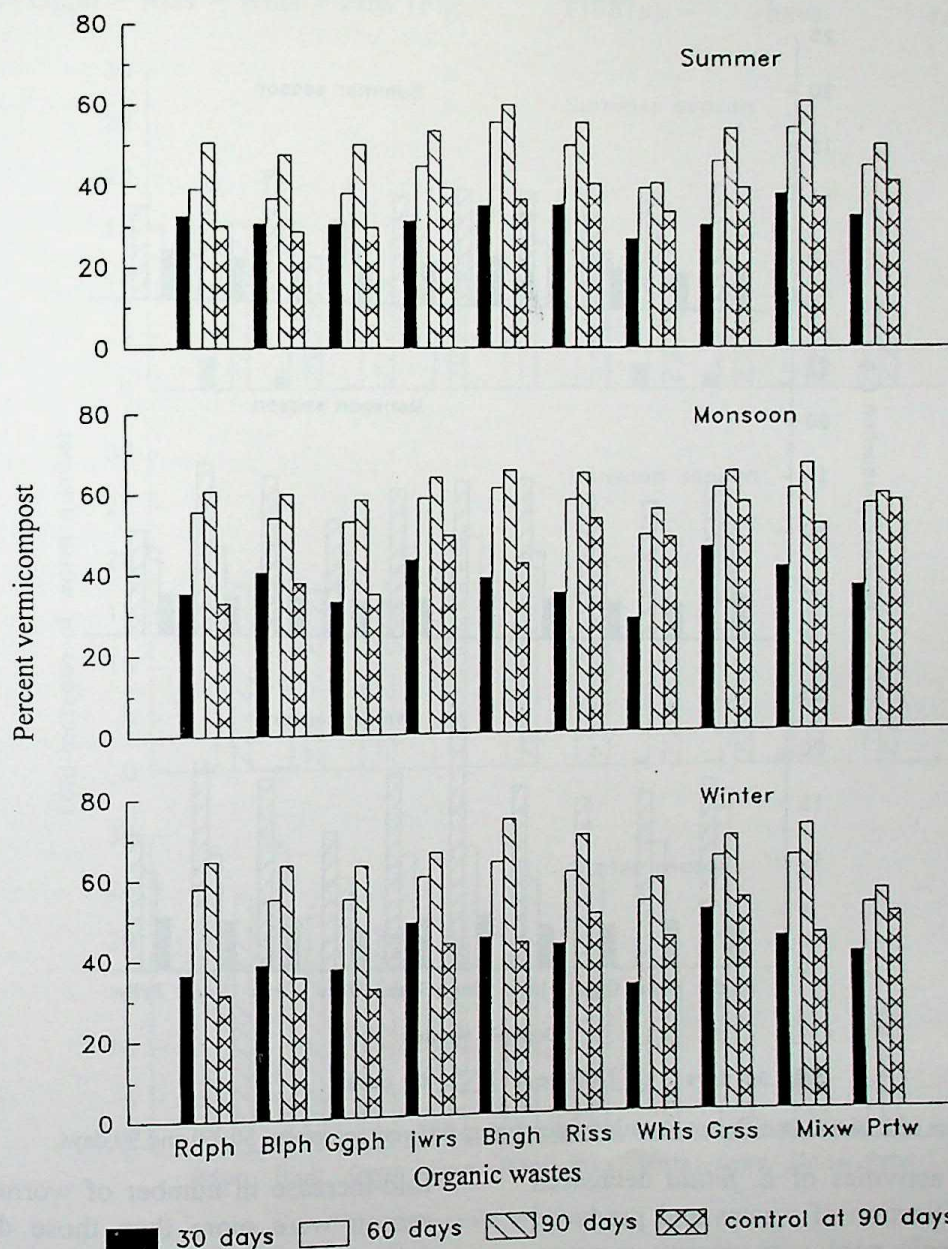


Fig. 1 : Percent vermicompost produced by *E. fetida* in different organic wastes during different seasons at 30, 60, 90 days and at 90 days control.

The maximum percent vermicompost and compost produced at 90 days during winter, monsoon and summer respectively were 73.82% (Bngh) and 53.18% (Grss), 66.76% (Mixw) and 57.24% (Grss), and 61.60% (Mixw) and 40.86% (Riss). The influence of seasonal factors on

degradation of wastes revealed that the compost (without worms) production was enhanced in monsoon > winter > summer, while vermicompost production was high in winter > monsoon > summer in all organic wastes. The amount of compost produced during 90 days,

during different seasons was more than the vermicompost produced during 30 days. Enhanced degradation of wastes in the presence of worms might be due to their feeding activity.

Variations in amount of vermicompost production with the waste diets were attributed to the quality and availability of them (Krishnamoorthy, 1986) and the seasonal factors (Lavelle *et al.*, 1993).

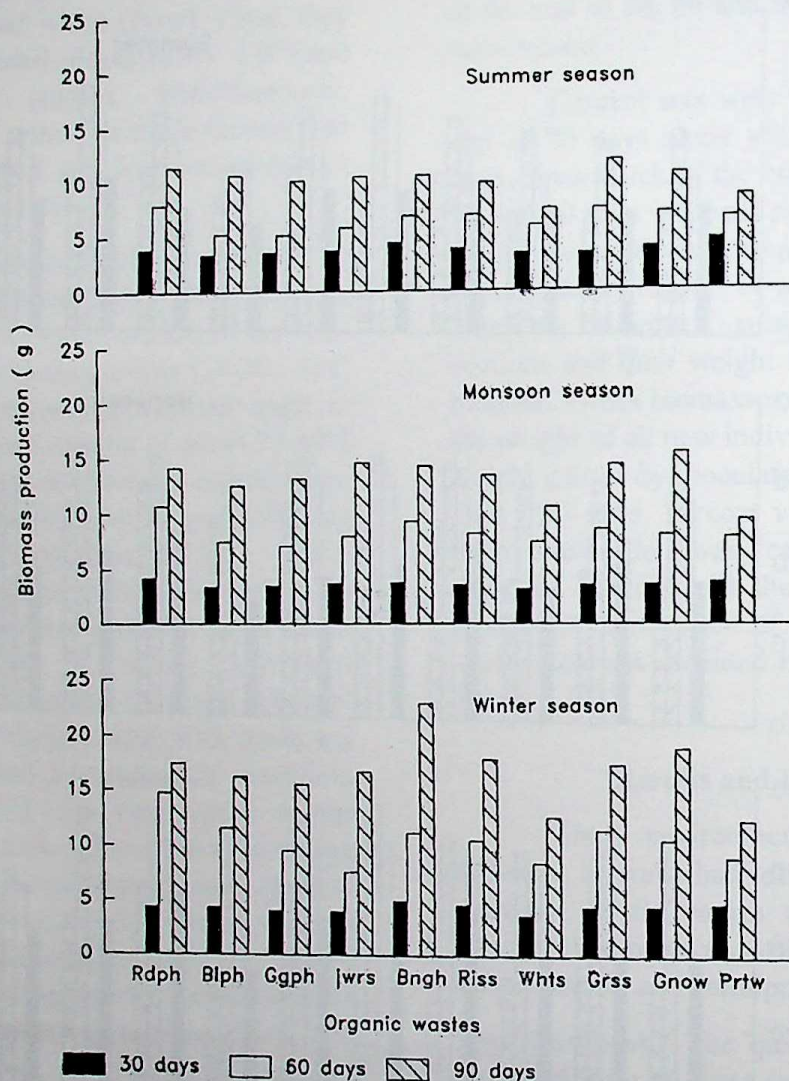


Fig. 2 : Gross biomass of *E. fetida* in different organic wastes during different seasons at 30, 60 and 90 days.

Feeding activities of *E. fetida* decreased the time of stabilization of wastes and produced an efficient organic pool with energy reserves. This ability of the worm depended very much on the nature of wastes from which they were derived and the seasonal factors.

Vermiprotein : Biomass of inoculated worms increased from initial weight to almost double in all diets during different seasons. In all amended diets, the gross biomass (including cocoons, juveniles, sub-clitellates and new clitellates) and

fold-increase in number of worms during winter season were more than those during monsoon followed by summer season (Figs. 2 and 3). The reproductive potential of the worm was affected by not only the quality and availability of food but also by the environmental conditions. Amoji *et al.* (2000) have also reported enhanced growth and reproduction of *E. fetida* during winter season.

Gross biomass production during winter was in Bngh > Mixw > Riss > Grss > Jwrs >

Compostability and production of vermiprotein by E. fetida.

Rdph > Blph > Ggph > Whts > Prtw. It in monsoon was Mixw > Bngh > Rdph > Grss > Jwrs > Blph > Riss > Ggph > Whts > Prtw and in summer it was Rdph > Grss > Mixw > Bngh > Jwrs > Blph > Ggph > Riss > Whts > Prtw (Fig.

2). The variation in biomass production in different diets during different seasons may be due to their nutritional status and favorable environmental factors. Kale and Krishnamoorthy (1981a) have reported that

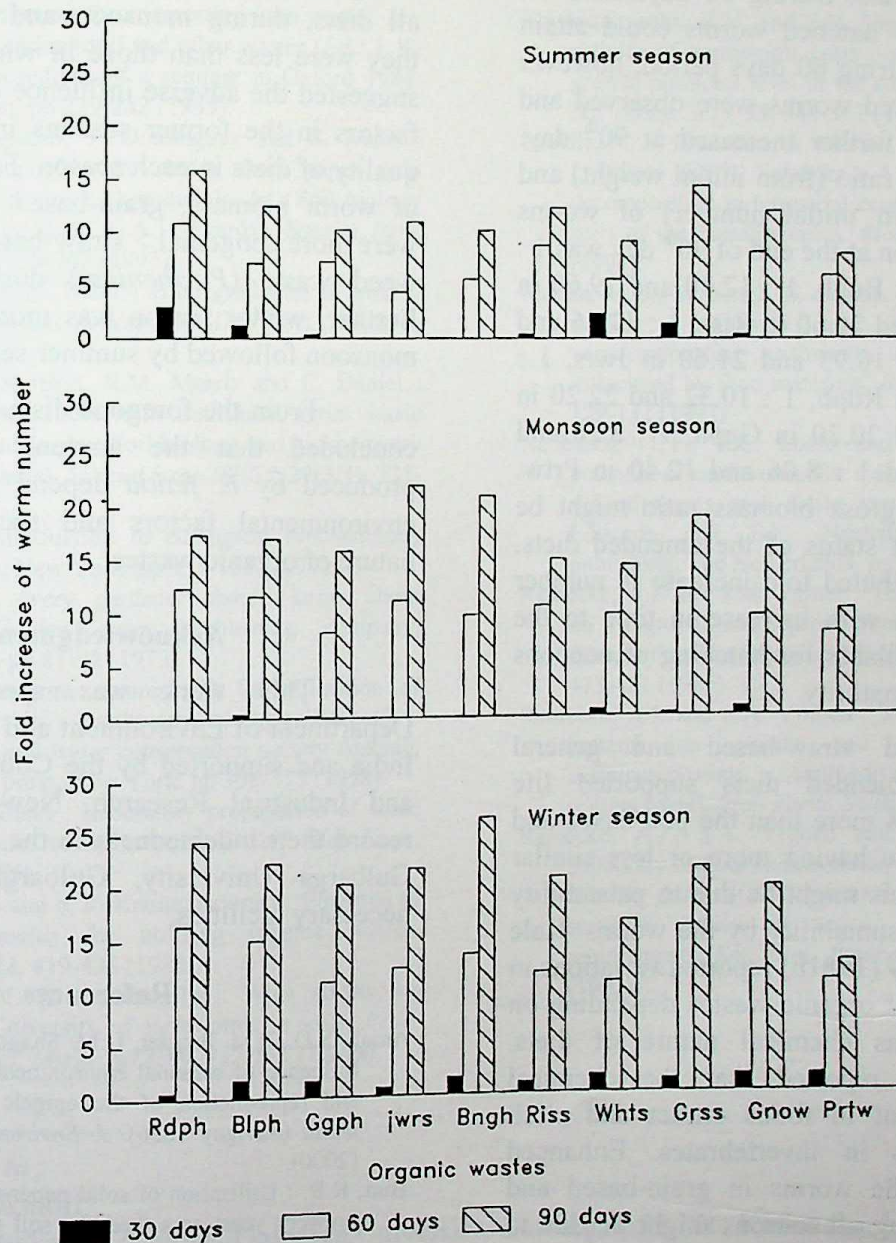


Fig. 3 : Number fold increase of *E. fetida* in different organic wastes during different seasons of 30, 60 and 90 days.

the nature of available food influences worm activity. In all diets during different seasons there was significant correlation between increase in worm number and gross biomass production with increase in days. This might be due to increased degradation of organic matter with time,

providing more form of nutrients that are available to worms resulting into enhancement in the reproductive capability and hence increased number and biomass of the worms. Reinecke and Venter (1985), and Reinecke and Viljoen (1990)

have also reported the increase in biomass with the feeding activity of the worms.

In all diets, during different seasons there were always more number of cocoons > juveniles > sub-clitellates > new clitellates during 30, 60 and 90 days intervals. During 30 days' interval, none of the newly hatched worms could attain sexual maturity. During 60 days period, however a few newly matured worms were observed and their number was further increased at 90th day. The gross biomass ratio (from initial weight) and fold increase (from initial number) of worms during winter season at the end of 90th day was 1 : 15.14 and 26.40 in Bngh, 1 : 12.40 and 19.60 in Mixw, 1 : 11.63 and 20.60 in Riss, 1 : 11.46 and 21.40 in Grss, 1 : 10.93 and 21.60 in Jwrs, 1 : 10.19 and 24.40 in Rdph, 1 : 10.32 and 22.20 in Blph, 1 : 9.95 and 20.20 in Ggph, 1 : 8.28 and 16.40 in Whts, and 1 : 8.06 and 12.40 in Prtw. The variations in gross biomass ratio might be due to the nutrient status of the amended diets. New juveniles attributed fold increase in number (Fig. 3) of worms with increase in time to the length of time available for hatching of cocoons and attainment of maturity.

Grain and straw-based and general organic waste amended diets supported life activities of worms more than the pod-based and weed diets, despite having more or less similar nutritive status. This might be due to palatability of wastes and consumability by the worms. Kale and Krishnmoorthy (1981b) reported variations in the acceptability of organic wastes depending on texture as well as chemical nature of diets. Harborne (1977) reported that the chemical constituents present in foods attract and elicit feeding responses in invertebrates. Enhanced reproduction of the worms in grain-based and general diets during all seasons might be due to the presence of more protein and variety of chemical constituents in them. The moderate life activities of the worms observed in pod-based diets could be attributed to the presence of tannin/lignin, which might have rendered them unpalatable for the worms, as was suggested, by Hopp (1973) and Lavelle *et al.* (1993). The least life activities of worms in Prtw could be due to

either its low nutritive status or the inhibitory influence of the 'parthenin' substance present in it. Apart from quality of diet, the life activities of the worms were influenced by the environmental factors. Gross biomass and fold number of worms though, increased from 30 to 90 days' intervals in all diets, during monsoon and summer season; they were less than those in winter season. This suggested the adverse influence of environmental factors in the former seasons in addition to the quality of diets in each season. For the production of worm biomass, grain-based and mixed diets were more congenial > straw-based > pod-based > weed waste (*Parthenium*) during all seasons. Further, winter season was more congenial than monsoon followed by summer season.

From the foregone discussion, it could be concluded that the amount of vermiprotein produced by *E. fetida* depend primarily on the environmental factors and secondarily on the nature of organic wastes.

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Utilization of banana agricultural waste: Production of cellulases by soil fungi.

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Abstract : Banana a major cash crop of Maharashtra is cultivated over 46900 hectares generating large amount of agro waste after the harvest. Attempts were made to utilize these agro wastes for production of cellulases. Of the 127 fungi isolated from the soil of banana field, 12 fungi were found to utilize cellulose as source of carbon. *Trichoderma lignorum* showed appreciable cellulolytic activity. It produced C_1 , C_x and β glucosidase in Carboxymethyl Cellulose Peptone medium as well as on agro waste based medium containing leaves, stem and rhizome powders. *T. lignorum* (0.45U/ml) produced maximum enzymes on leaf based medium

Key words : Banana plant waste, *Trichoderma lignorum*, Cellulases.

Introduction

Celluloses, Hemicelluloses, Pectin, starch etc. form bulk of agricultural wastes. Hydrolysis of these substrates yields fermentable sugars that can be processed further as chemical feedstock or fuel. Conversion of these wastes to compost had been reported by many workers (Subbarao, 1982; Catton, 1983). The recent stress on renewable energy resources demands a proper utilization of these waste generation understanding of role of microbial enzymes in biodeterioration. A number of fungi such as species of *Trichoderma reesei* (Reese *et al.*, 1950), *Chaetomium cellulolyticum* (Chahal and Hawksworth, 1976), *Aspergillus versicolor* (Deshpande and Gurucharanam, 1985), *Penicillium sp.*, *Myrothecium sp.* (Reddy *et al.*, 1998) etc. have been reported to possess cellulolytic activity. The search for new cellulolytic organisms continues.

Banana is major cash crop of this region. In Maharashtra, it occupies an area of 46900 hectare with an average production of 53.95 ton /hectare (Anonymous, 2000). After the harvest of the fruits the completely plant-leaves, stem and rhizome is left in the field for natural degradation, which takes months together.

However, these wastes can be utilized for cellulase production. In the present paper, the suitability of these wastes for use as carbon source in the medium has been defined. Utilizing the agro waste available in the region and isolating the soil fungi for the synthesis of cellulolytic enzymes had been attempted.

Materials and Methods

Organism : The organisms used in the study were isolated from soil (Black Cotton) of the fields at Nanded, Maharashtra. Soil samples were collected from the fields where Banana was cultivated as ratoon crop for continuously seven years. Fungi were isolated and enumerated by serial dilution method after growth on the Carboxymethyl Cellulose (CMC) Peptone and Banana waste base medium. The fungal isolates were identified comparing the characteristic with already described ones (Domsch *et al.*, 1980).

Substrate : The initial screening and selection was made by their ability to utilize Carboxy methyl Cellulose (CMC) in the CMC Peptone Medium. CMC : 10 gm. Peptone : 2.5 gm. KH_2PO_4 : 1 gm. $MgSO_4$: 0.5 gm. Distilled water : 1000 ml. pH : 5.5.

Agricultural waste based medium was used for isolation of fungi and production of cellulolytic enzymes. CMC was replaced by dried finely powdered (40 mesh – 120 μ) leaf waste, stem and rhizome separately. The dried leaves, the stem and the rhizome were used as substrate for the production of cellulases. The leaves, stem and rhizome were washed thoroughly with water, dried in the oven at 70°C and grinded into fine powder. This powder was supplemented to the basal medium.

Cultural studies : The isolated fungus was grown in Erlenmeyer flasks (250 ml) containing the liquid medium (50 ml) and then were autoclaved at 20 lbs for 20 min. The flasks containing medium were inoculated with 5-mycelial disc (7 mm dia) punched out from the edges of its 8 days old colonies in petriplates. The flasks were incubated at $27 \pm 2^\circ\text{C}$ for 10 days. The culture filtrate was collected, centrifuged at 2000g for 30 minutes to remove all spores. The supernatant was filtered through Whatman no.1. This preparation was used as crude cellulolytic enzyme solution during the course of study.

Cellulases activity : Assay for cellulases was done following method of Bergham and Petterson (1973) with slight modifications. For C_1 enzyme : 100 mg of filter paper dust was suspended in 1 ml of 0.01 M Sodium acetate buffer of pH 4.8 was incubated with 2 ml of crude enzyme solution. For C_x enzyme : 2ml of crude enzyme solution was mixed with 4 ml of CMC and 1ml of the 0.01 M Sodium acetate buffer of pH 4.8 and were incubated at $27 \pm 2^\circ\text{C}$.

Aliquots were drawn from the mixture at regular time interval and the release of glucose due to the enzyme activity was assayed by 3,5 Dinitro salicylic acid method (Miller, 1959) using D glucose as standard.

Assay method for β glucosidase : Activity of β glucosidase was assayed by the method of Eberhart *et al.*, (1963) using p-nitro β glucoside as substrate. The reaction mixture consist of 50 mg of p-nitro β glucoside in 2 ml of 0.01 M acetate buffer at 4.8 pH and 1 ml crude enzyme preparation incubated at $27 \pm 2^\circ\text{C}$. At regular time

intervals aliquots were drawn and added with 0.1 N NaOH and the release of p-nitro phenol from substrate was estimated for absorbance at 420 nm in a spectrophotometer.

Soluble reducing sugars (equivalent to glucose) were released from filter paper was estimated and the cellulase activity was expressed in enzyme units. Enzyme activity is expressed in Units (U). One enzyme unit is equivalent to amount of enzyme required to release 1 μ mole of D glucose (for C_1 and C_x) or P nitro phenol (For β glucosidase) per minute from respective substrate.

Results and Discussion

The fungal population in the soil from the banana field is shown in the Table 1. Soil samples from the field yielded *Aspergillus niger*, *A. flavus*, *Curvularia lunata*, *Alternaria tenuis*, *Fusarium roseum* and *Trichoderma lignorum* on petriplates. The fungal cultures isolated from the soil were identified by comparing the characteristic from already reported ones. *Trichoderma lignorum* was dominant fungus colony with 28% appearing on plates. Therefore, it was preferred for the study of enzyme secreted by *T. lignorum*. The dominance on the medium indicates the relative secretion of cellulases by the fungi. This indicates the suitability of substrate for the secretion of cellulases. The utilization of glucose from the substrate as sole carbon source can be correlated with the ability of cellulases secretion. (Reese, 1956)

T. lignorum in liquid cultures produced cellulases with CMC as well as banana biomass as carbon source. It can be concluded that production of cellulases complex can be achieved using banana biomass as source of carbon designing a cheap medium containing simple constituent. However, it is clear from the Table 2 banana powders of leaf, stem and rhizome were effectively utilized as carbon source with slight difference in the production of cellulases. *T. lignorum* showed highest activity on dried leaf powder closely followed by stem powder whereas it was least with that of rhizome powder. However, the cellulases secretion on the leaves was slightly more than on CMC medium. In

natural habitat availability of a single substrate as carbon source is not guaranteed. Thus, cellulose and other polysaccharides in any selected habitat would be contaminated with other carbohydrates,

minerals, growth factors etc. Enzyme production under these conditions would certainly be better, whenever additional nutrient are available. This can be attributed to the presence of mineral and

Table - 1 : Quantitative distribution of cellulolytic fungi in the soil from banana fields.

Fungi	CMC medium		Banana leaf medium	
	Nos. of colonies/gm X 1000	% appearance	Nos. of colonies/gm X 1000	% appearance
<i>Aspergillus niger</i>	15	12.39	28	15.68
<i>A. flavus</i>	11	09.08	15	08.4
<i>A. fumigatus</i>	07	05.78	07	03.92
<i>Alternaria tenuis</i>	06	04.95	11	06.16
<i>A. humicola</i>	07	05.78	11	06.16
<i>Chaetomium sp.</i>	05	04.13	03	01.68
<i>Cladosporium</i> <i>herbarum</i>	06	04.95	13	07.28
<i>Curvularia lunata</i>	07	05.78	11	06.16
<i>Fusarium roseum</i>	04	02.47	09	05.04
<i>Penicillium</i> <i>citrinum</i>	05	04.13	11	06.16
<i>Trichoderma</i> <i>lignorum</i>	35	28.92	39	21.84
Non-sporulating form	13	10.74	24	13.94

Table - 2 : Production of cellulases by *Trichoderma lignorum* on CMC and banana waste based medium.

Time (Days)	Cellulase activity (U/ml)											
	Banana leaf medium			Banana stem medium			Banana rhizome medium			CMC medium		
	C _i	C _x	β glucosidase	C _i	C _x	β glucosidase	C _i	C _x	β glucosidase	C _i	C _x	β glucosidase
3	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00
4	0.02	0.10	0.03	0.03	0.07	0.01	0.01	0.04	0.00	0.03	0.08	0.02
5	0.05	0.19	0.07	0.04	0.14	0.06	0.03	0.09	0.01	0.05	0.18	0.07
6	0.09	0.26	0.12	0.08	0.20	0.10	0.06	0.14	0.05	0.09	0.23	0.11
7	0.14	0.31	0.13	0.11	0.27	0.12	0.08	0.19	0.08	0.12	0.29	0.13
8	0.15	0.39	0.14	0.13	0.33	0.11	0.10	0.25	0.10	0.14	0.36	0.13
9	0.15	0.45	0.13	0.12	0.39	0.10	0.10	0.29	0.09	0.14	0.42	0.12
10	0.13	0.42	0.12	0.11	0.38	0.10	0.08	0.28	0.10	0.12	0.41	0.11

other nutrients present in the leaves followed by stem and rhizome. The source of carbon used in growth medium of cellulolytic fungi has two effects. It stimulate the growth and enzyme production or only growth is favored with reduction of enzyme activity (Reese and Mandel, 1960)

Thus, a plant biomass in a simplified medium supplemented with simple salts proved to cost effective substrate for cellulase production as

well as cellulolytic hydrolysis. This may be of practical importance in large-scale enzymatic hydrolysis of lignocellulosic plant waste during bio-ethanol production.

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Study of physico-chemical characteristics of water bodies around Jaipur.

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Abstract : The present study has been undertaken to evaluate physico-chemical parameters (pH, temperature, dissolved oxygen, free carbon dioxide, alkalinity and hardness) and zinc concentration in water bodies in and around Jaipur. Water samples from Jalmahal Lake, Nevta Lake, Amer Lake and Ramgarh Lake were analysed. Results reveal that the water of Jalmahal Lake is most polluted due to high pH, hardness, alkalinity, free carbon dioxide, zinc content, and a low level of dissolved oxygen. Contrarily Ramgarh Lake is least polluted, as it has high dissolved oxygen and low pH, alkalinity, free carbon dioxide, hardness and zinc content.

Key words : Hydrobiology, Zinc concentration.

Introduction

Water pollution is a major problem in many parts of the country. It is increasing day-by-day around industrial and urban centres. Limnological studies of Lakes and reservoirs have been carried out by Singh and Mahaveer (1997) and Jain and Srivastava (1998) but their correlation to heavy metal content and their impact on physiology of fish have seldom been made.

Zinc is an essential trace element in living organisms. It has a cumulative and persistent action (Alabaster and Llyod, 1982). It is involved in nucleic acid synthesis and stabilizes the structure of DNA, RNA and ribosomes (Mc Dowell, 1992). Zinc also participates in a variety of metabolic processes involving carbohydrates, lipids, proteins and nucleic acids (Vallee, 1988). However, changes in blood parameters (Singh, 1995; Banerjee, 1998) and tissue structure (Gupta and Chakrabarti, 1995) have been reported on exposure to zinc. Therefore, zinc can be toxic when present in excess amounts. Physico-chemical parameters of water affect water quality individually and in combination with zinc. Variations of physico-chemical parameters also have a direct bearing on the structure and functions of various organs (Khillare and Dovane,

1998; Dhanapakiam *et al.*, 1998). Hence monitoring of zinc and evaluation of physico-chemical parameters in water bodies is important. The present paper, therefore, deals with the evaluation of physico-chemical parameters and zinc concentrations of water bodies around Jaipur. Their impact on physiological functions will subsequently be studied.

Materials and Methods

Water samples in glass bottles were collected from four different Lakes i.e. Jalmahal Lake, Amer Lake, Nevta Lake and Ramgarh Lake situated on the outskirts of Jaipur city.

Atmospheric and water temperature were measured on sites. Water samples for dissolved oxygen were fixed on sites in BOD bottles. Physico-chemical parameters i.e. hardness, alkalinity, free carbon dioxide and dissolved oxygen were estimated according to APHA, AWWA and WPCF (1989) and expressed as mg/L. Amount of zinc present in water was estimated by Atomic Absorption Spectrophotometer.

Results and Discussion

The level of heavy metals in water bodies greatly influences the lives of biota therein.

Results of the present investigation revealed that the values of zinc concentration in water are in decreasing order from Jalmahal > Amer > Nevta and Ramgarh Lake. Values of zinc concentrations

in Jalmahal, Amer, Nevta and Ramgarh are 0.152 mg/L, 0.136 mg/L, 0.123 mg/L and 0.116 mg/L, respectively.

Table - 1 : Zinc concentrations (mg/L) and physico-chemical characteristics (mg/L) of water bodies.

S. No	Parameters	Jalmahal Lake	Amer Lake	Nevta Lake	Ramgarh Lake
1.	Zinc concentration	0.152 ± .03	0.136 ± .04	0.123 ± .04	0.116 ± .01
2.	Atmospheric temperature	Max. 31.0°C	Max. 31.0°C	Max. 32.0°C	Max. 31.0°C
3.	Water temperature	Min. 15.0°C	Min. 15.0°C	Min. 18.0°C	Min. 18.0°C
4.	PH	23.5°C	24.0°C	22.8°C	22.6°C
5.	Hardness	9.0	8.0	8.0	7.5
6.	Dissolved oxygen	173.0 ± 1.24	166.25 ± 1.19	159.0 ± 0.471	157.70 ± 0.552
7.	Free Carbon dioxide	4.76 ± 0.221	5.70 ± 0.11	6.36 ± 0.221	7.606 ± 0.188
8.	Phenolphthalein alkalinity	12.656 ± 1.01	9.53 ± 0.733	6.05 ± 0.635	Nil
9.	Total alkalinity	235.0 ± 3.33	186.0 ± 1.05	182.25 ± 3.13	164.5 ± 0.333
		420.0 ± 4.714	354.0 ± 2.82	324.0 ± 1.24	270.0 ± 4.71

Values are mean ± standard deviation).

Background values of zinc in natural inland surface waters are reported to vary generally from 0.001 to 0.2 mg/L (O' Connor, 1968).

The safe levels of discharge of zinc, on inland surface water as given by ISI (1982) and drinking water standards as given by WHO (1971) and ISI (1982) is 5 mg/L. The National Acad. of Science for irrigation water, however, has given 2.0 mg/L for long-term use and 10.0 mg/L for short-term use (Abbasi and Soni, 1986). The levels of zinc observed in water bodies in the present investigation are, therefore, well within the permissible limits and hence these bodies are not toxic for the biota therein.

Temperature is one of the most important ecological factors, which controls the physiological behaviour, and distribution of organisms. Shakar *et al.* (1993) and Jain *et al.* (1996) have observed diurnal variations in temperature. In the present study, diurnal variations were noted and water temperature was found to be lower than atmospheric temperature at all the four sites. Water temperature of Amer showed highest values and Ramgarh reservoir showed the lowest.

During the present study, pH value ranged from 7.5 to 9.0; it was 7.5 at Ramgarh and 9.0 at Jalmahal. This is in accordance with earlier

reports by Wetzel (1972) who reported that the value of pH ranges from 8.0 to 9.0 units in Indian waters. Mary Bai (1989) has observed that the values of pH of the polluted stations ranged from 8.0 to 9.0 whereas the pH of unpolluted stations was 8.0 at palar river (Karnataka). Keeping in view the pH, the water samples at Ramgarh appear to be less polluted than the samples at Jalmahal. This fact is also corroborated by the dissolved oxygen content recorded at these sites. It was 7.6 mg/L at Ramgarh and 4.7 mg/L at Jalmahal. Mary Bai (1989) reported that the dissolved oxygen at the unpolluted stations varied from 8.82 to 9.6 mg/L. Jain *et al.* (1989) observed that the dissolved oxygen shows an inverse relation with water temperature at all station of Halali reservoir of Vidisha district (M.P.). Similar correlation can be observed in the present study where relatively higher dissolved oxygen is observed in Lakes having lower water temperature (Ramgarh and Nevta Lakes).

In the present investigation, the values of carbon dioxide vary from 6.05 mg/L to 12.65 mg/L and are not within detectable limits (almost nil) at Ramgarh Lake. Jain *et al.* (1996) estimated nil to 5.6 mg/L of carbon dioxide at Halali reservoir. Similarly, Mathew (1978) observed an inverse relationship between carbon dioxide and dissolved oxygen in Govind Sagar Lake. A

similar relationship is also observed in the present study where dissolved oxygen is found highest at Ramgarh while CO₂ is nil. Present work is in agreement with the report of Pandey and Soni (1993) who had observed high values of free carbon dioxide, alkalinity and pH along with low dissolved oxygen in highly polluted Lake water at Naukuchiyatal Lake situated in Kumaon, Himalayas. In the present work, lowest D.O. is recorded at Jalmahal (4.7 mg/L) with high CO₂ (12.65 mg/L) and high total alkalinity (420 mg/L). The reverse is observed at Ramgarh with highest dissolved oxygen (7.6 mg/L), absence of CO₂ and lower values of total alkalinity (270 mg/L). Trivedi and Goel (1992) pointed out that dilution plays an important role in lowering alkalinity of water. Lower values of alkalinity at Ramgarh may be related to this factor, since Ramgarh Lake is a larger Lake in comparison to Jalmahal and Amer Lakes. Pandey and Sony (1993) observed higher amounts of total alkalinity at highly polluted Naukuchiyatal Lake.

Observations of the present study revealed that the values of hardness are in decreasing order from Jalmahal > Amer > Nevta > Ramgarh. Lloyd (1960) has reported that high values of hardness increase toxicity of zinc to fish; the level of zinc at Jalmahal was recorded to be highest in the present study and could in turn be more toxic for fishes.

The present findings indicate that the water of Jalmahal Lake is most polluted due to high alkalinity, free carbon dioxide, hardness, pH, zinc content, and a low level of dissolved oxygen. Contrarily Ramgarh Lake appears to be least polluted amongst all the Lakes studied, since it has high dissolved oxygen, low alkalinity, free CO₂, hardness, pH and zinc content.

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Decolorization of anthraquinone dye by *Aspergillus ficuum* in various physiological states.

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Abstract : Decolorization of reactive brilliant blue KN-R by *Aspergillus ficuum* was investigated on suspended spores, mycelial pellets, immobilized cells. It was found that *Aspergillus ficuum* could effectively decolorize reactive brilliant blue KN-R especially when grown as pelleted mycelia. Many factors affecting the decolorization process in nitrogen-limited media (NLM) were studied, including : initial pH, temperature, and mycelial age. Results showed that the media containing reactive brilliant blue KN-R at 50mg/L could be decolorized by 96% of the initial color in 42h, in most conditions tested, the dye degraded products assayed by UV-visible spectrophotometer and macroscopic observation showed that the decolorization of reactive brilliant blue KN-R by mycelial pellets includes two important processes : biodegradation and biosorption. Kinetic study revealed that reactive brilliant KN-R biodegradation by mycelial pellets and suspended spores conformed to first-order reaction model while reactive brilliant blue KN-R biodegradation by immobilized cell followed zero-order model. In addition, mycelial pellets was found to biodegrade KN-R more quickly than suspended spores and immobilized cell.

Key words : *Aspergillus ficuum*, Decolorization, Anthraquinone dye, Effluent treatment, Kinetics.

Introduction

Synthetic dyes are widely used in the field of printing and dyeing of textiles, paper printing, and leather and as additives in petroleum products. Over 7×10^5 tons and approximately 10,000 dyes are produced annually worldwide, of which about 10% is lost in the industrial effluents (Vaidya and Datye, 1982). Color is usually recognized as the first contaminant in wastewater. Even a very small amount of dye in water (10-50 mg/L) is highly visible and affects the aesthetic merit, water transparency and the gas solubility of waterbodies. Color removal from wastewater is often more important than the removal of soluble colorless organic substances which usually contribute the major factor of chemical or biochemical oxygen demand (Banat *et al.*, 1996).

Azo, anthraquinone and indigo are three major chromophores of various commercial dyes (Rattee, 1995). Many microbes have been found affective to azo dye decolorization. Wong and Yuan (1996) had reported the decolorization and biodegradation of the azo dye methyl red under

aerobic conditions using *Klebsiella pneumoniae* sp. Coughlin *et al.* (1997) reported the aerobic azo dye biodegradation with several strains capable of using the azo dye as the sole source of carbon energy and nitrogen. Much of the work undertaken dye degradation concerned the decolorization of azo dyes (Harmer and Bishop, 1992; Ganesh *et al.*, 1994; Seshadri *et al.*, 1994). Most studies in the area of dye degradation dealt with azo dyes, however, few were reported on the decolorization ability of fungi on anthraquinone. In this paper, the reactive brilliant blue, a kind of anthraquinone, was applied in the decolorization tests by *Aspergillus ficuum* of various physiological statuses. Then the effect of physical and chemical parameters on the decolorization as well as the kinetics of the biodegradation of KN-R by *Aspergillus ficuum* was also reported.

Materials and Methods

Fungi and material : *Aspergillus ficuum* was isolated from sludge of Wenzhou printing plant. It was maintained on plates of Sabouraud Dextrose Agar and stored at 4°C. Mycelial pellets growth

media was 4.0. Sabouraud dextrose agar broth (1L) with glucose 40 g, peptone 10 g in pH 4.0 constituted mycelial pellet growth media. The solution (1L) composed of Ammonium tartrate 0.22 g, Glucose 10.0 g, KH_2PO_4 2.0 g, MgSO_4 0.5 g, CaCl_2 0.1 g, α -ketoglutaric acid 1.08 g, vitamin B_1 1 mg, Tween 80 0.1% 50 ml, pH 4.0 was prepared for decolorizing tests. The decolorization tests were done with anthraquinone dye reactive brilliant blue KN-R (Fig. 1), Dye : C.I. reactive brilliant blue KN-R was from Donggang Group, Jiangsu, China.

Cultivation of mycelial pellets : The mycelial inoculum was prepared by homogenizing fungal mycelium aseptically. Erlenmeyer flasks (250 ml) containing 100 ml of Sabouraud Dextrose Agar were inoculated with 5 mL of the spores suspension (2.8×10^7) and incubated at 33°C on a rotary shaker (150 r/min) for 2 days. The mycelial pellets were harvested after cultivation and then used in the decolorization tests.

Immobilization of biomass : When continuously stirring to avoid formation of lumps, 8 g of alginate sodium salt was dissolved in 400 ml of hot distilled water and thus Alginate solution of 2% was ready. The slurry was cooled to room temperature and equal amount of suspended spores were added under stirring condition to have a uniform mixture. This mixture was extruded as droplets in 50 mM CaCl_2 solution, using peristaltic pump. The gel beads were allowed to cure for 24h at 33°C and washed thoroughly with distilled water.

Table – 1 : Degradation of reactive brilliant KN-R by various states.

State	Kinetics equation	K ($\text{mg}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$)	r^2
Mycelial pellet	$\ln c = -0.07211 t + 3.9423$	0.07211	0.9746
Suspended spore	$\ln c = -0.06741 t + 4.2346$	0.06741	0.9471
Immobilized cell	$\ln c = -0.6779 t + 51.02$	0.6779	0.9560

suspended spores, mycelial pellets, immobilized cells respectively, and was incubated at 30°C , 150 r/min for 42h. In this period, the optical density (OD) of samples was measured at certain intervals. The results (Fig.2) showed that the mycelial pellets have the highest decolorization

Decolorization of dye : Mycelial pellets, alginate beads with biomass and suspend spores were respectively inoculated to media for decolorizing tests in Erlenmeyer flasks at 33°C , 150 r/min, for 42h. The optical density (OD) of samples before and after decolorization was measured (at λ_{max} 594 nm) spectrophotometrically using 751 GW UV-visible spectrophotometer. All experiments were repeated for three times. The rate of decolorization was calculated according to the following formulation :

$$\text{Rate of decolorization (\%)} = \frac{[\text{OD}_{(\text{before decolorization})} - \text{OD}_{(\text{after decolorization})}]}{\text{OD}_{(\text{before decolorization})}} \times 100\%$$

In each experiment, the initial biomass of *Aspergillus ficuum* was always very similar in all the cultures as shown by the close similarity of replicates. Typically most experiments had : 5 ml suspended spores at a concentration of 2.8×10^7 per milliliter, mycelial pellets and immobilized cells, the same amount as the suspended spores.

Results and Discussion

Effect of various physiological state of *Aspergillus ficuum* on reactive brilliant blue KN-R decolorization : To determine the effect of various physiological state of *Aspergillus ficuum* on reactive brilliant blue KN-R decolorization, 50 ml media containing reactive brilliant blue KN-R decolorization (The final dye concentration was 50 mg/L) was inoculated with the same amount of

ability. In the first 24h, the suspended spores showed much lower decolorization ability than the mycelial pellets. This may be due to ascribed to the process of spores sprouting and mycelial growth. However, in the subsequent period they demonstrated equal decolorization ability.

However, the mycelial pellets offer many advantages over suspended spores because of its certain size, mechanical intensity and convenience for practical practices. Immobilized cells have the lowest decolorization ability. *Aspergillus ficuum*, as an aerobic microbe, is difficult to get oxygen and substrate. Certain decolorization rate took place during the first 12h because blank alginate beads showed certain adsorption (Volesky *et al.*, 1995).

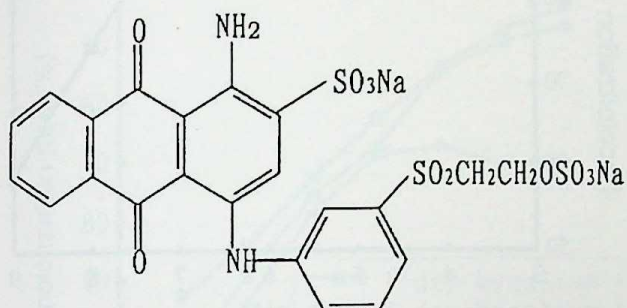


Fig. 1 : The chemical structure of reactive brilliant blue KN-R.

Kinetics of reactive brilliant blue KN-R biodegradation : For the *Aspergillus ficuum* in three physiological statuses, dye concentration change with time in the experiments is illustrated (Fig.3). It is well known that biodegradation of microbe is due to decolorizing enzyme. The equation of biochemical reaction rate is :

$$\gamma = \frac{\gamma_m \cdot c}{\kappa + c} \quad (1)$$

Where r is reaction rate, γ_m is the maximal reaction rate, c is substrate concentration, κ is a constant of semi-saturation rate. If $c \ll \kappa$, equation (1) could be rewritten as :

$$\gamma = \frac{\gamma_m \cdot c}{\kappa} \quad (2)$$

It implies that the biodegradation follows the first-order model, and the rate constant is :

$$\kappa_1 = \frac{\gamma_m}{\kappa}$$

If $c \gg \kappa$, equation (1) could be rewritten as :

$$\gamma = \gamma_m \quad (3)$$

It implies that the biodegradation follows the zero-order model, and the rate constant is :

$$\kappa_0 = \gamma_m$$

According to Eq. (2) and Eq. (3), the relation between the substrate concentration c and time is obtained, and described as the first-order reaction equation and the zero-order reaction equation respectively :

$$\ln c = a + k_1 t$$

$$c = b + k_0 t$$

The constants K (κ_1 and κ_0) were calculated for the biodegradation of reactive brilliant blue KN-R by the *Aspergillus ficuum* in three physiological states (Table 1).

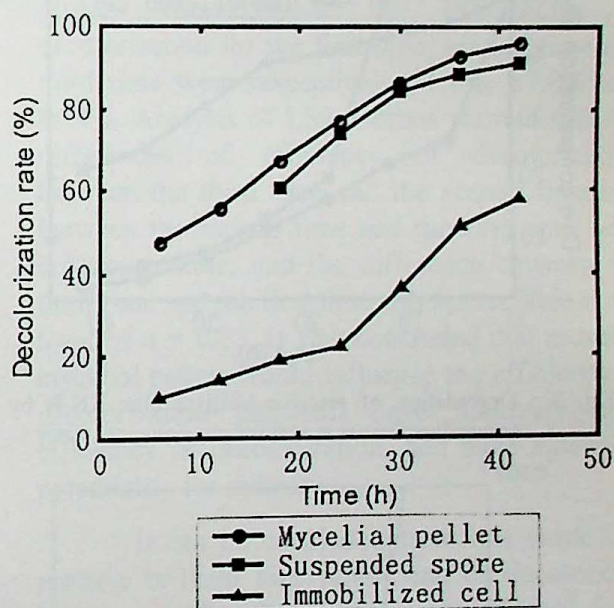


Fig. 2 : Effect of various physiological state of *Aspergillus ficuum* on reactive brilliant blue KN-R decolorization.

The result showed that the biodegradation follows the first-order model when *Aspergillus ficuum* is in the states of mycelial pellet and suspended spore, and in the state of immobilized cell, the zero-order model. It was demonstrated that reactive brilliant KN-R could be biodegraded more quickly by mycelial pellets than by suspended spores and immobilized cells.

Bioadsorption and biodegradation : In this experiment, the color of mycelia pellets turned from white to blue after 12h when they were inoculated to the media containing reactive brilliant blue KN-R, which changed from blue to

white blue. In the next 30h, however, the media turned into white green. The macroscopic observation showed that the decolorization of reactive brilliant blue KN-R by mycelial pellets includes two important processes : bioadsorption and biodegradation. The specific peak (at 594 nm) of reactive brilliant blue KN-R in absorption spectra had completely disappeared and there was a sharp

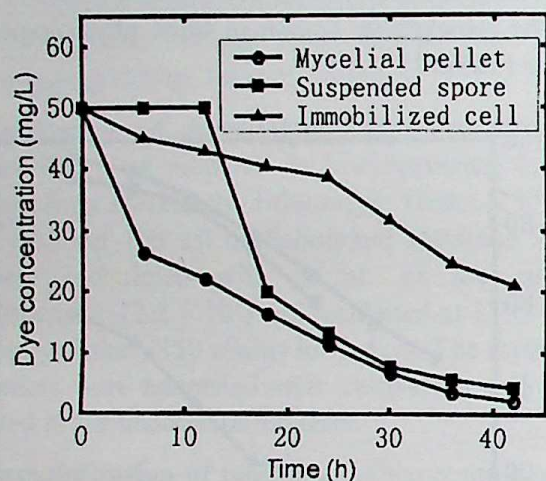


Fig. 3 : Degradation of reactive brilliant blue KN-R by *Aspergillus ficuum* at various physiological states

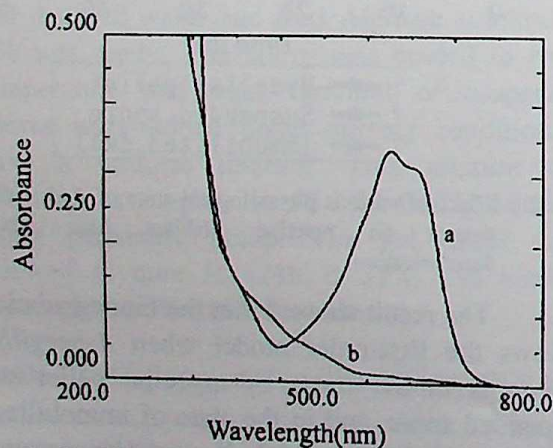


Fig. 4 : The UV-visible spectra of reactive brilliant blue KN-R before and after decolorization by *Aspergillus ficuum*.

a : Before decolorization
b : After decolorization

increase in low UV light absorption. This result showed that the molecular structure of reactive brilliant blue KN-R had been changed after decolorization and that biodegradation really happened (Fig.4).

Effect of culture condition of mycelial pellets on reactive brilliant blue KN-R decolorization : To determine the effects of agitation on the decolorization process, mycelial pellets were used

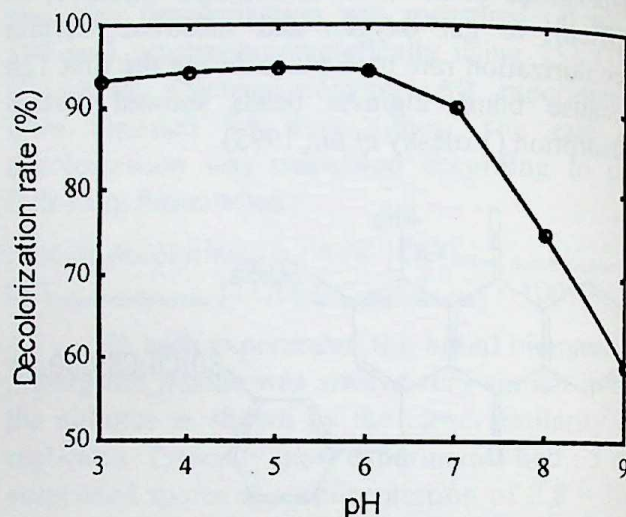


Fig. 5 : The effect of pH on decolorization of reactive brilliant blue KN-R.

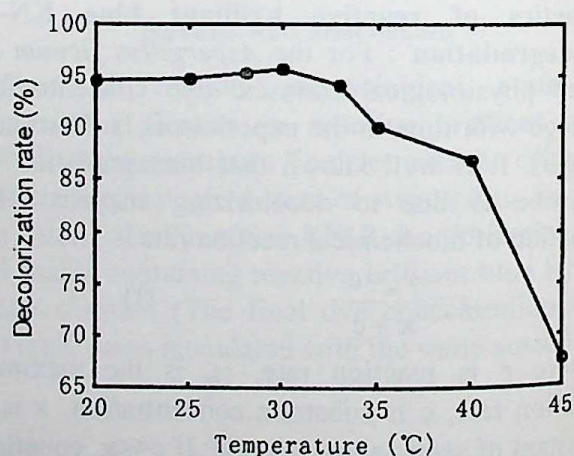


Fig. 6 : The effect of temperature on decolorization of reactive brilliant blue KN-R.

in the static and shaken cultures, after 42h incubation in static conditions there was only 56.80% decolorization of 50 mg/L reactive brilliant blue KN-R whereas in shaken conditions 96% decolorization occurred. Thus, agitation was very effective in improving the rate of decolorization by mycelial pellets. This may be because of improved mass transformation of oxygen and substrates in shaken cultures.

To determine the effect of initial pH and to establish the optimum initial pH for decolorization, a series of media differing only in initial pH was tested. The results (Fig.5) showed that reactive brilliant blue KN-R was not easily decolorized by mycelial pellets when initial pH was greater than value 7. Media with initial pH values ranging from 3.0 to 6.0 all gave similarly good results, so the optimum pH is 5.0.

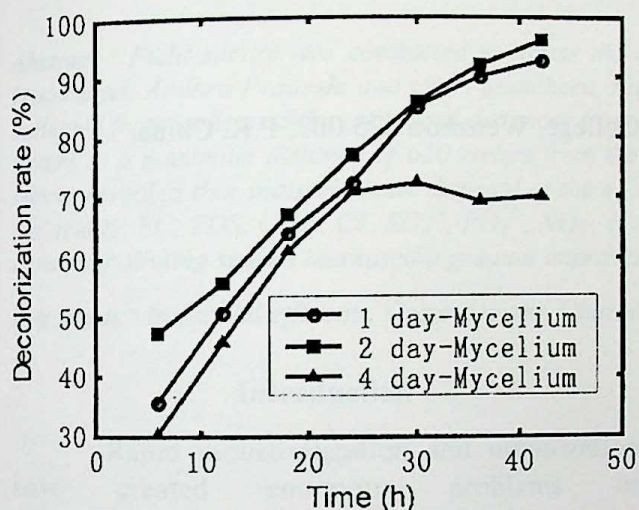


Fig. 7 : The effect of mycelia age on decolorization of reactive brilliant blue KN-R.

Decolorization of reactive brilliant blue KN-R by *Aspergillus ficuum* in NLM was carried out at various temperatures (20-45°C). Decolorization ability was stable at the range of 20-35°C and was maximal at 30°C, while at 45°C it was relatively low (Fig.6). The reason may be that under high temperature the enzyme in *Aspergillus ficuum* became inactive. The ability to decolorize reactive brilliant blue KN-R during primary mycelial growth was investigated. Two kinds of media were used for mycelial growth and decolorization-Sabouraud Dextrose Agar and NLM. The media containing reactive brilliant blue KN-R were inoculated with homogenized mycelium. The initial decolorization rate in NLM was higher than that in Sabouraud Dextrose Agar. But the amount of mycelium in the Sabouraud Dextrose Agar was more than that in NLM (dry weight of mycelia : 1.2 g/L and 0.5 g/L respectively).

Mycelial pellets cultured at different periods in Sabouraud Dextrose were tested for their decolorization ability. Mycelium grown for 2 days demonstrated the highest decolorization rate, while those grown for 4 days performed the lowest decolorization rate. (Fig.7)

Test of re-decolorization of mycelial pellets :

Mycelial pellets that had decolorized in 50 ml of 50 mg/L medium for 42h were taken out and added into another same medium containing dye, the efficiency of decolorization were detected after decolorizing in the same condition. The results showed that the efficiencies of decolorization for the first time, second time and third time were respectively 91.1%, 87.8% and 86.4%. Analysis of LSR method showed that the differences of efficiency of decolorization between the third time and the second time and between the second time and the first time were not appreciable; and the difference between the third time and the first time was appreciable at the level of $\alpha = 0.05$. It was concluded that reusable mycelial pellets would influence the efficiency of decolorization but still have relatively high efficiency of decolorization, and have extensive potentiality for industry.

It can be concluded from this work that reactive brilliant blue KN-R can be decolorized by *Aspergillus ficuum* in each of the three physiological states. A modified first-order reaction analysis, could account for mycelial pellets decolorizing the dye at a faster rate than suspended spores. Thus, mycelial pellets maybe the best the physiological state of *Aspergillus ficuum* for the removal of dye from aqueous effluent.

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Effect of industrial effluent on properties of groundwater.

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Abstract : Field survey was conducted to assess the quality of underground water in four major industrial areas of Hyderabad, Andhra Pradesh, and viz., Patancheru, Katedhan, Nacharam and Jeedimetla. Ground water samples were collected from wells at different lateral distances from the effluent streams i.e., starting from a minimum distance of 20 meters to a maximum distance of 620 meters from the effluent streams and analysed for different characteristics. The survey revealed that indiscriminate disposal of the effluent of industrial complexes around Hyderabad has aggravated the acidity, EC, TDS, COD, Cl^- , SO_4^{2-} , PO_4^{3-} , NO_3^- , F and heavy metals in the ground waters. As the sampling distance from the polluting stream increased a gradual improvement in the quality of ground water was noticed.

Key words : Industrial effluents, Ground water, Characteristics.

Introduction

Rapid industrialization and urbanization have created enormous problems of environmental pollution in terms of generating the variable quantity and quality of effluents. Untreated and contaminated industrial effluents increased the concentration of Cd, Pb, Zn, Cu and Mn in nearby water bodies (Totawat, 1993). The information available is mainly related to municipal and/or a specific type of industrial effluents, but most of the drains in industrial area carry the mixed effluent of various factories. However, the studies on this aspect are lacking. Keeping this in view, the present investigation was undertaken to assess the effect of mixed effluent on ground water quality in the four major industrial areas of Hyderabad viz., Patancheru, Katedhan, Nacharam and Jeedimetla.

Materials and Methods

Patancheru, Katedhan, Nacharam and Jeedimetla are major industrial areas in Hyderabad of Andhra Pradesh. Cement factories, Dying units, Batteries manufacturing units, cycle factories etc., are situated in those areas discharge their treated effluents of varying quality into a

main drain which flows along the agricultural fields. The drain carrying the mixed effluent is not well maintained and effluent usually spread over the neighboring agricultural fields and wells. Effluent samples (30) from the various points at mid depth of the drain as well as ground water samples (36) from wells were collected at different lateral distances from the effluent streams along with control (water samples collected from non polluted area). Farmers have been using the ground waters from the wells located in the adjoining areas of the effluent streams for irrigation since several years. In view of this surface soil samples (0-15 cm depth) were collected at different lateral distances from effluent streams as that of ground water collection, in order to find the effect of their use on soil properties. A surface soil sample from non-polluted area was collected for the purpose of comparison. Water and effluent samples were analysed for pH, Electrical conductivity (EC), Total dissolved solids (TDS), Chemical oxygen demand (COD), Carbonates (CO_3^{2-}), Bicarbonates (HCO_3^-), Chlorides (Cl^-), Sulphates (SO_4^{2-}), Phosphates (PO_4^{3-}), Nitrates (NO_3^-), Fluoride (F), Zinc (Zn), Iron (Fe), Manganese (Mn), Copper (Cu), Cadmium (Cd), lead (Pb), Nickel (Ni), Chromium (Cr) and Cobalt (Co) as per methods

outlined by American Public health Association (1985). Micronutrients and heavy metals were determined by using Atomic Absorption Spectrophotometer. The soil samples were analysed for pH, EC and organic carbon (Jackson, 1973). Available nitrogen was determined by alkaline permanganate method (Subbiah and Asija, 1956), available phosphorus by Olsen's method (Olsen et al., 1954) and available potassium by neutral normal ammonium acetate method (Jackson, 1973).

Results and Discussion

Chemical composition of industrial effluents : Characteristics of the effluent (Table 1) vary over a wide range and this is quite expected as the effluents come from all sorts of industries. Analysis of industrial effluents collected from the four major industrial areas of Hyderabad have clearly indicated very high contents of all chemical constituents such as EC, TDS, COD, Cationic and anionic forms of nutrients.

Table - 1 : Chemical composition of the mixed industrial effluent.

Characteristic	Patancheru range	Katedhan range	Nacharam range	Jeedimetla range
pH	2.3-8.2	4.5-8.0	3.1-8.4	2.8-8.3
EC (dSm ⁻¹)	0.98-6.24	1.12-4.82	0.86-7.64	0.78-6.82
TDS (mg l ⁻¹)	280-1400	310-1100	480-1560	410-1380
COD ((mg l ⁻¹)	1.6-46.8	1.0-21.2	1.3-28.2	1.8-24.6
Cl ⁻ (mg l ⁻¹)	110-868	98-628	130-860	122-910
Co ₃ ²⁻ (mg l ⁻¹)	5.2-10.8	4.1-8.3	3.0-9.2	5.8-12.0
Hco ₃ ⁻ (mg l ⁻¹)	25.8-50.4	20.8-44.8	18.5-48.6	27.5-63.5
So ₄ ²⁻ (mg l ⁻¹)	77-862	62-710	59-785	68-740
Po ₄ ³⁻ (mg l ⁻¹)	0.8-8.0	0.5-7.3	0.9-6.6	0.4-7.0
No ₃ ⁻ (mg l ⁻¹)	3.8-16.4	3.0-14.8	4.2-15.6	2.2-12.6
F (mg l ⁻¹)	0.5-3.5	0.3-2.8	0.2-3.8	0.4-4.1
Zn (mg l ⁻¹)	0.08-2.9	Traces-1.5	0.05-2.8	0.07-1.8
Fe (mg l ⁻¹)	0.35-5.5	1.05-6.5	1.5-5.8	0.50-4.5
Mn (mg l ⁻¹)	0.50-3.2	Traces 2.5	0.7-3.5	1.0-4.1
Cu (mg l ⁻¹)	Traces -2.1	Traces -1.7	0.5-1.4	Traces-1.5
Cd (mg l ⁻¹)	Traces-1.2	Traces- 0.8	Traces-1.7	Traces-0.7
Pb (mg l ⁻¹)	Traces-2.5	Traces 0.6	Traces-1.3	Traces-1.1
Ni (mg l ⁻¹)	Traces-1.8	Traces -1.2	0.1-2.8	0.05-2.0
Cr (mg l ⁻¹)	Traces-0.8	Traces -1.6	Traces-3.1	Traces-1.8
Co (mg l ⁻¹)	Traces-1.1	Traces-0.8	Traces-1.6	Traces-1.0

Quality of ground water : The data on the analysis of ground waters collected from the wells located at different lateral distances from the polluting stream at the four sampling sites viz., Patancheru, Katedhan, Nacharam and Jeedimetla (Table 2) have clearly indicated that the ground waters have been adversely effected by polluting effluent streams when compared to WHO (1984) standards for drinking water (Table 3) or as per the quality of normal well water collected from non polluting environment. The deterioration in the quality was reflected in all the parameters including pH, EC, TDS, Cl⁻, CO₃²⁻, HCO₃⁻, SO₄²⁻, PO₄³⁻, NO₃⁻, F and heavy metals.

Effects of ground water on human health : The polluting effluent streams changed the ground water towards the acidic range, increased the total concentration of soluble salts EC, TDS, COD, SO₄²⁻, PO₄³⁻, NO₃⁻, F and heavy metals, which have adverse effect on the quality parameters of the ground waters. Deterioration of groundwater quality, due to industrial effluents has been reported by Muller (1985).

According to WHO (1984) the limit for chloride in drinking water is 250 mg l⁻¹ based on taste consideration. Chlorides in drinking water are not generally harmful to human beings although for

people suffering from disease of heart or kidney; high chloride in drinking water is considered as unsafe. Near the stream ground waters, contain

relatively higher Cl^- content when compared to away from the stream. SO_4^{3-} has also shown the similar trend.

Table - 2 : Range and Mean of properties of ground waters collected under the polluting effluent streams.

Property	Normal well	Patancheru range			Katedhan range			Nacharam range			Jeedimetla range		
		Near the stream	Away from the stream	Mean \pm SE	Near the stream	Away from the stream	Mean \pm SE	Near the stream	Away from the stream	Mean \pm SE	Near the stream	Away from the stream	Mean \pm SE
PH	7.4	3.1	7.8	6.9 0.45	5.8	7.8	7.1 0.27	3.8	7.8	6.9 0.47	3.2	7.8	7.0 0.51
EC (dsm ⁻¹)	0.81	4.94	1.76	2.96 0.30	4.14	1.65	2.76 0.31	6.81	1.21	3.40 0.68	6.42	1.34	3.05 0.68
TDS (mg/l ⁻¹)	230	1100.0 0	1.80	538.00 102.14	820.00	210.00	486.00 80.98	1420.00	325.00	692.00 25.91	1270.0 0	320.00	714.00 100.69
COD (mg/l ⁻¹)	1.20	32.00	1.40	9.20 2.83	11.10	1.10	5.60 1.41	24.00	0.90	9.30 2.47	20.00	1.00	6.40 2.09
Cl^- (mg/l ⁻¹)	54.00	756.00	91.00	375.00 54.88	574.00	72.00	282.00 61.92	762.00	112.00	361.00 81.03	748.00	104.00	336.00 73.54
CO_3^{2-} (mg/l ⁻¹)	3.40	2.80	6.8	4.62 0.43	3.10	8.80	5.48 0.70	2.10	10.40	5.87 1.19	4.40	9.40	6.31 0.54
HCO_3^- (mg/l ⁻¹)	15.60	12.70	34.5	21.83 2.40	18.20	38.40	29.65 2.64	11.05	46.35	28.90 4.96	18.50	43.20	29.85 2.65
SO_4^{2-} (mg/l ⁻¹)	36.20	758.00	57.00	360.00 79.93	595.00	45.00	265.00 69.10	698.00	48.00	366.00 72.21	668.00	41.00	322.00 71.91
PO_4^{3-} (mg/l ⁻¹)	0.30	4.00	0.40	2.10 0.33	3.30	0.30	1.70 0.37	3.60	0.50	1.80 0.36	3.20	0.20	1.30 0.36
NO_3^- (mg/l ⁻¹)	1.30	11.40	2.00	8.59 0.81	10.20	1.50	5.60 1.13	10.80	1.80	5.50 1.05	9.40	1.40	5.10 0.98
F (mg/l ⁻¹)	0.10	1.85	0.10	0.60 0.16	1.05	0.13	0.53 0.12	2.00	0.13	0.87 0.24	1.75	0.20	0.71 0.17
Zn (mg/l ⁻¹)	0.01	0.57	0.02	0.20 0.06	0.43	0.01	0.15 0.05	0.62	0.03	0.29 0.07	0.56	0.06	0.32 0.06
Fe (mg/l ⁻¹)	0.08	2.47	0.11	0.79 0.23	2.12	0.08	0.77 0.24	2.26	0.26	0.93 0.23	1.98	0.18	0.89 0.21
Mn (mg/l ⁻¹)	0.01	1.01	0.02	0.46 0.13	0.76	0.04	0.37 0.09	0.81	0.05	0.38 0.08	0.76	0.07	0.42 0.08
Cu (mg/l ⁻¹)	ND	0.71	ND	0.24 0.07	0.52	ND	0.22 0.07	0.62	0.01	0.25 0.07	0.82	0.01	0.25 0.09
Cd (mg/l ⁻¹)	ND	0.020	0.001	0.007 0.002	0.016	ND	0.005 0.002	0.051	ND	0.016 0.006	0.034	ND	0.008 0.004
Pb (mg/l ⁻¹)	ND	0.150	0.008	0.059 0.010	0.051	0.004	0.026 0.006	0.078	0.010	0.031 0.007	0.064	0.008	0.032 0.007
Ni (mg/l ⁻¹)	0.011	0.098	0.007	0.029 0.008	0.052	0.002	0.026 0.006	0.076	0.081	0.044 0.007	0.068	0.014	0.034 0.006
Cr (mg/l ⁻¹)	0.003	0.028	0.002	0.011 0.003	0.038	0.003	0.017 0.004	0.071	0.002	0.027 0.007	0.040	ND	0.015 0.004
Co (mg/l ⁻¹)	0.002	0.037	0.004	0.015 0.003	0.027	0.001	0.011 0.003	0.022	ND	0.008 0.003	0.020	ND	0.009 0.002

There are some substances whose presence above the limit cause ill effects on health of humans and animals. Among those substances, first important one is Fluoride. Excessive fluorine in drinking water produces

dental fluorosis. F content in the ground waters near the effluent stream was $> 1 \text{ mg/l}^{-1}$ which is slightly higher as per the WHO (1984) standards for drinking water.

In some circumstances, nitrates in the drinking water have been shown to cause health hazard to infants and possibly elder children, if the concentration in drinking water is $> 45 \text{ mg l}^{-1}$ because after reducing to nitrite in the circulatory system, they may cause methemoglobinemia. Levels of NO_3^- in the groundwaters were within the safer limit as per WHO (1984) standards for drinking water.

Among the heavy metals, concentration even after 617 mt. distance from the effluent stream in Patancheru was higher than the limits prescribed by WHO (1984) for drinking water. Pb level in the ground waters was within the safe limit in the very first well itself in Katedhan, Nacharam and Jeedimetla locations while at Patancheru location it was above the safer limit even at 103 mt from the effluent streams. Higher levels of Pb in

Table - 3 : Standards for drinking and irrigation water.

S. No.	Characteristic	WHO (1984) drinking water	ISI 2296 (1982) irrigation water
1	pH	6.5 - 8.5	6.0 - 8.0
2	EC (micromhos cm^{-1})	--	2.25
3	TDS (mg l^{-1})	1000	2,100
4	Cl^- (mg l^{-1})	250	600
5	SO_4^{2-} (mg l^{-1})	400	1000
6	NO_3^- (mg l^{-1})	10	--
7	F (mg l^{-1})	1.5	--
8	Zn (mg l^{-1})	5.0	--
9	Fe (mg l^{-1})	0.3	--
10	Mn (mg l^{-1})	0.1	--
11	Cu (mg l^{-1})	1.0	--
12	Cd (mg l^{-1})	0.005	--
13	Pb (mg l^{-1})	0.05	--
14	Cr (mg l^{-1})	0.05	--
15	B (mg l^{-1})	--	2.0

drinking water cause tiredness, lassitude, irritability, anemia and behavioural changes in children. Cr content was within the safe limit and became safer as per the standards prescribed by WHO (1984) for drinking purpose. Higher levels of Cr have been implicated as the cause of cancer of the digestive tract in man.

Effect of industrial effluents on quality of ground water for irrigation : The total concentration of soluble salts expressed as electrical conductivity in ground waters irrespective of sampling distance under the four polluting environments of Patancheru, Katedhan, Nacharam and Jeedimetla was higher than the limits prescribed by ISI (1982) for irrigation purpose (Table 3) thereby indicating the more adverse effect of higher soluble salts in the effluent streams. Chlorides have also shown the similar trend. This indicated build up of high salt

concentration in the well waters is due to the industrial effluents. Similar increase in concentration of soluble salts, and other chemical constituents in well waters due to effluent contamination have been reported by Ashok Kumar et al., (1998).

Interestingly, ground waters contained relatively higher quantities of SO_4^{2-} , PO_4^{3-} , NO_3^- , and micronutrients such as Zn, Fe, Mn and Cu (Table 2), which have positive role as plant nutrients. It is well known that along with constituents deleterious to the quality of ground waters, the polluting effluent streams depending on the nature of the industry from which the effluent emanates, some of the ions having nutritional importance in plant growth are also incidentally added. This trend appears to have happened at irrespective of sites of industrial area under study. The increase in concentration of

some of the plant nutrients like SO_4^{2-} , PO_4^{3-} , NO_3^- , in water bodies with factory effluents have been reported by Bhatt and Pathak (1992).

Effect of distance from effluent stream on quality of ground water : Distance within which a particular property of ground water became to be within the safer limit, changed among the different polluting environments of Patancheru, Katedhan, Nacharam and Jeedimetla. The distance, within which the property attained the safer limit, as per the WHO (1984) norms were

summarised in Table 4. These data clearly indicated that the percolating polluted effluent water was being reduced in different kinds of salts and ions, through a process of ion exchange between the percolating waters and soil colloids on one hand and through various kinds of chemical reactions with the other soil constituents, which were changing from location to location. Variation in the chemical properties as per the WHO (1984) norms in the wells nearer to

Table - 4 : Safer distance for property/constituent of ground water under the polluting effluent streams (m).

Property/Constituent	Patancheru	Katedhan	Nacharam	Jeedimetla
pH	206	63	62	31
EC	*	*	*	*
TDS	227	139	175	381
COD	*	377	309	515
Cl^-	494	176	113	361
$\text{SO}_4^{=}$	412	176	227	381
$\text{PO}_4^{=}$	*	377	*	515
NO_3^-	206	50	41	1 st well
$\text{CO}_3^{=}$	227	139	175	381
HCO_3^-	206	63	113	361
F	206	63	113	361
Zn	1 st well	1 st well	1 st well	1 st well
Fe	412	289	309	381
Mn	412	377	309	515
Cu	412	289	227	381
Cd	206	50	113	155
Pb	103	1 st well	1 st well	1 st well
Ni	494	289	*	*
Cr	1 st well	1 st well	62	1 st well
Co	*	377	309	381

* Higher than safer limit even at farthest sampling, site.

the stream itself, except under Nacharam polluting effluent stream after a distance of 62m, reflect more of variations in the constitution of polluting effluent streams.

It clearly indicates as the sampling distance from the polluting streams increased; there was gradual improvement in the quality of ground waters.

Physicochemical properties and macronutrient status of soils : Physico chemical properties of soils (Table 5) showed that the soils located nearer to the effluent stream were acidic and

became either neutral or alkaline in pH as the sampling distance increased from the effluent stream. Mean total soluble salt content was very high in polluted soils (1.99 to 2.64 dSm^{-1}) when compared to non-polluted soil (0.38 dSm^{-1}). Mean organic carbon content in polluted soils varied from 0.32 to 0.50% while in non polluted soil it was only 0.26% . Nitrogen, Phosphorus and Potassium contents were high in polluted soils and decreased with distance from the polluting stream.

High concentration of nitrates and phosphates in the ground waters nearer to the

polluting streams appears to have contributed for the higher amounts of those nutrients in the soil as compared to those located away from the stream. It is also possible that changes in the fertility management practices and cropping, have also contributed for the same.

It can be concluded from the present investigation that the chemical parameters such as concentration of soluble salts, TDS, COD, CO_3^{2-} , HCO_3^- , SO_4^{2-} , PO_4^{3-} , NO_3^- , F and heavy metals exceeds slightly in ground waters in four major

Table - 5 : Physico chemical properties and macronutrient status of surface soil samples collected under the polluting effluent stream.

Property	Normal soil	Patancheru		Katedhan		Nacharam		Jeedimetla	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
pH	7.4	5.8-8.2	7.4	6.2-8.0	7.5	5.6-7.8	7.3	5.7-7.8	7.2
EC (dSm^{-1})	0.38	0.88-4.66	2.61	0.92-4.08	2.64	0.75-3.98	1.99	0.85-3.74	2.16
Or C (%)	0.26	0.24-0.54	0.36	0.21-0.46	0.32	0.30-0.66	0.50	0.28-0.60	0.45
N (Kg ha^{-1})	211	228-570	382	206-465	353	290-660	494	273-590	460
P_2O_5 (Kg ha^{-1})	20	20-60	31	17-48	29	10-52	26	11-49	26
K_{20} (Kg ha^{-1})	258	303-980	498	281-760	490	170-968	475	141-840	440

Normal soil = Soil collected from non-polluted area.

industrial areas of Hyderabad viz., Patancheru, Katedhan, Nacharam and Jeedimetla as per the norms of WHO (1984) when compared with normal water. The increase in concentration of chemical constituents was more in the wells nearer to the effluent streams when compared to wells at about $\frac{1}{2}$ km away from the effluent stream. Soil samples also showed the similar trend in case of total soluble salts concentration and macronutrient status.

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Biodiversity of algae and protozoa in a natural waste stabilization pond : A field study.

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Abstract : A field study was carried out on the biodiversity of protozoa and algae from a natural waste stabilization pond during November, 1996 to April, 1997. The raw waste and pond samples were analysed for physico-chemical and biological parameters. High dissolved oxygen (DO) coinciding with phytoplankton peak was recorded. The algae - *Chlorella vulgaris*, *Scenedesmus acuminatus*, *Oscillatoria brevis* and *Nostoc piscinale* and Protozoa - *Paramecium caudatum*, *Acanthamoeba* sp., *Bodo saltans* and *Oikomonas termo* were obvious as dominant species, whereas algae *Ochromonas pyriformis* and *Synura uvella* and protozoa, *Didinium masutum* and *Stentor coerulus* were noted as rare species. Totally 71 species of algae and 13 species of protozoa were identified.

Key words : Waste stabilization pond, Algae, Protozoa, BOD and catalase.

Introduction

Stabilization pond is a simple scientifically designed pit with 2-6 feet depth, where BOD reduction takes place by supporting algal-bacterial growth (Hosetti *et al.*, 1985). These ponds are commonly used in warm climates to purify wastewater. The performance of pond depends on climatological conditions like light, temperature, rain, wind and the wastewater quality. The bacteria in the pond decompose the biodegradable organic matter and release carbon dioxide, ammonia and nitrates. These are utilized by the algae, together with sunlight and photosynthetic process releases oxygen, enabling the bacteria to breakdown waste and accomplish reduction in BOD levels (Pearson *et al.*, 1987).

The waste stabilization ponds considered as the secondary treatment methods, by which natural process of purification and stabilization is accelerated. The ponds efficiently remove bacteria, biodegradable organics and reduce the combined phosphorous and nitrogen to be discharged to the receiving streams (Hosetti and Rodgi, 1985).

In most places of India, the wastewater is not regulated properly; often it enters into low-

lying areas and through ponding natural purification occurs. In the places where designed ponds are not available, the wastes allowed to flow through natural channels and ponds, the effluents are reused for irrigation. In the present study, also the wastes produced in the University Gent's Hostel are allowed to flow through an open channel. The channel ends in a waterfall, which formed a natural pond. The water samples from the open channel and the natural ponds are evaluated for the efficiency of waste treatment as a unit of open channel and natural pond system. Additionally the biodiversity of algae and protozoa in samples both in open channel and natural pond was estimated.

Materials and Methods

Raw wastewater samples from the channel (S₁) and from the natural waste stabilization pond (S₂) were collected once at 30 days interval for a period of six months from November 1996 to April 1997. The samples were analysed for pH, dissolved oxygen (DO), Biological Oxygen Demand (BOD₅), Phosphate (PO₄) and Total Dissolved Solids (TDS) according to the procedures given in APHA (1995). Algal counts were made based on

Hemocytometer cell counts using Lugol's Iodine solution. Protozoa were enumerated by using single drop method. Catalase activity was determined according to Hosetti and Frost (1994).

Statistical evaluation of the results included calculation of arithmetic mean, standard deviation and performing the regression analysis at a significant level of $P = 0.05$ in order to evaluate the hypothesis.

Results and Discussion

The various physico-chemical characteristics of raw sewage flowing through the open channel (S_1) and that of pond effluent (S_2)

are presented in Tables 1 and 2. The predominance of algae and protozoa species in S_1 and S_2 are shown in Tables 3 and 4 respectively. Monthly variations in algae density in S_1 and S_2 showed in Fig. 1 and 2 and monthly variations in protozoa density in S_1 and S_2 are shown in Fig. 3 respectively.

The characteristics of raw wastewater (S_1) flowing in the channel was as follows:

The pH ranged from 7.1 to 7.4, DO ranged from 1.0 to 1.4 mg/l, BOD ranged from 271 to 339.6 mg/l, PO_4 ranged from 5.6 to 8.7 mg/l, Catalase in the range of 19.8 to 26.7 units and the TDS was in the range of 535 to 598 mg/l

Table - 1 : Monthly variations in pH, DO and PO_4 of the sewage of open channel and natural pond.

Parameters	pH		DO (mg/l)		PO_4 (mg/l)	
Samples	S_1	S_2	S_1	S_2	S_1	S_2
November	7.1	9.3	1.1	12.8	5.6	2.1
December	7.2	9.4	1.3	14.9	7.7	2.6
January	7.3	9.0	1.0	14.4	8.7	3.6
February	7.2	8.9	1.0	12.2	8.1	4.8
March	7.3	9.2	1.4	13.1	7.3	2.0
April	7.4	9.5	1.0	15.8	5.6	2.2
Average \pm SD	7.2 ± 0.1	9.2 ± 0.2	1.1 ± 0.1	13.8 ± 1.3	7.1 ± 1.2	2.8 ± 1.1

S_1 - Sample from open channel

S_2 - Sample from natural waste stabilization pond

Table - 2 : Monthly variations in BOD, Catalase and TDS of the sewage of open channel and natural pond.

Parameters	BOD (mg/l)		Catalase*		TDS (mg/l)	
Samples	S_1	S_2	S_1	S_2	S_1	S_2
November	271.2	88.5	19.8	15.0	598	475
December	308.8	127.9	24.3	19.9	550	420
January	339.6	109.6	22.98	15.6	600	425
February	311.8	116.8	22.64	16.0	590	475
March	297.8	70.8	23.1	7.4	535	430
April	273.2	45.1	26.7	15.5	596	500
Average \pm SD	300.4 ± 25.8	93.1 ± 31.2	23.2 ± 2.2	14.9 ± 50	578.1 ± 28.2	454.1 ± 33.3

*Catalase Units = Micromoles of hydrogen peroxide decomposed per minute per 100 ml of sample.

S_1 - Open channel raw wastewater

S_2 - Sample from natural stabilization pond

(Tables 1 and 2). The total population density of algae and protozoa was in the range of 26.5 to

$48.2 \times 10^3/\text{ml}$ and 15.9 to $45.5 \times 10^3/\text{ml}$ respectively (Tables 3 and 4).

The physico-chemical characteristics of pond effluent (S_2) showed pH levels from 8.9 to 9.5, DO from 12.2 to 15.8 mg/l, BOD ranged

from 45.1 to 127.9 mg/l, PO_4 ranged from 2.1 to 4.8 mg/l, Catalase from 7.4 to 19.9 units and TDS from 420 to 500 mg/l respectively. The total

Table - 3 : List of algae encountered in open channel and natural waste stabilization ponds.

Cyanophyceae

Synochococcus elongatus
Merismopedia punctata
Gleocapsa turgida
G. punctata
Microcystis aeruginosa
Spirulina subsalsa
Oscillatoria redeckei
O. bravis
O. agardhii
Lyngbya martensians
Schizothrix calcicola
Microcoelus lyngbyus
Rivularia binsolettiann
Tolypothrix tenuis
Scytonema hofmannii

Aphanizomenon flosaquae

Nostoc piscinale

Anabaena oscillatoriodes

Chrysophyceae

Ochromonas pyriformis
Synura uvella

Bacillariophyceae

Coscinodiscus

Heridion Circulare

Diatoma vulgare

D. heimale

Antorionella formosa

Fragillaria capucina

F. Vaucheria

Achnanthes linearis

A. exigua

Neidium iridis

Pinnularia viridis

Navicula lanceolata

N. bacillum

Gyrosigma attenuatum

Cymbella minuta

Gomphonema olivaceum

Nitischia palea

N. communis

Euglenophyceae

Euglena acus

E. gracilla

E. viridia

E. mutabilis

Phacus

Trachelomonas volvocina

Chlorophyceae

Chlamydomonas gloeogemma

Chlorogonium elongatum

Pandorina morum

Eudorina elegans

Volvox aureus

Tetraspora gelatinosa

Pediastrum simplex

P. duplex

Ankistrodesmus falcatus

A. convolutes

A. gracilis

Chlorella vulgaris

Chlorococcum infusiorum

Scenedesmus acuminatus

S. armatus

S. quadricauda

Botryococcus braunii

Ulothrix zonata

Geminella minor

Microspora amoena

Cladophora glomerata

Spirogyra sp.

Zygnema eactinatuma

Closterium chrenbergii

Cosmarium circulare

C. ralfsii

C. malanosporum

population density of algae and protozoa was in the range of 128.3 to $240.8 \times 10^3/\text{ml}$ and 8.9 to $29.4 \times 10^3/\text{ml}$ respectively.

The rise in pH levels in natural waste stabilization pond (S_2) may be due to the microbial activity and hike in DO in pond (S_2) was due to the oxygenation by autotrophic algae (Ganapati, 1974; Haertel 1976; Laal *et al.*, 1994) and secondarily by surface aeration through diffusion of atmospheric oxygen into the water.

Considerable success has been achieved in waste stabilization ponds in the removal of essential nutrients like nitrates and phosphates (Gloyne, 1971). Amongst 40 to 44 micronutrients

required for algae, the phosphates and nitrates are referred as most essential nutrients. In the present context, the estimation of total phosphates was taken as a measure of nutrient status in the samples. The raw sewage contained an average of 7.1 mg/l of phosphate. It was reduced to about 60.6% in the effluents of natural waste stabilization pond. The phosphate removal may be due to precipitation of phosphate as hydroxyapatite ($Ca_5(PO_4)_3 OH$) at alkaline pH levels developed due to photosynthetic activity (Anonymous, 1973). The precipitation process leads to increase in the sedimentation rate. Part of the phosphates removed may also be due to anabolic uptake by the green algae (Curtis and

Mara, 1994). A significant correlation was observed between the percent removal of phosphate and the population density of algae protozoa in both the samples ($r = 0.47$ and 0.49 at $P = 0.05$ for algae and protozoa respectively in sample S_1 and $r = 0.29$ and 0.40 for algae and 0.40 at $P = 0.05$ for algae and protozoa in sample S_2) respectively.

The raw sewage meandering in the Channel (S_1) recorded average BOD value of 300.4 mg/l. After passing through the natural stabilization pond BOD was reduced to an average of 93.1 mg/l. The reduction in the BOD level was maximum in April and minimum in December. The average removal of BOD was 69% . Relatively low rate of BOD removal in

Table - 4 : Monthly variations in raw flowing sewage and effluent samples.

Protozoa	November		December		January		February		March		April	
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Sarcodina												
1. <i>Acathamoeba</i>	6,500	2,538	6,000	2,088	4,316	2,436	4,900	6,156	5,400	3,349	8,440	998
2. <i>Arcella vulgaris</i>	3,500	89	1,434	800	1,089	1,477	36	500	110	981	944	167
Mantogophora												
3. <i>Synura</i>	5,000	424	938	534	1,300	1,275	936	1,250	942	560	2,438	340
4. <i>Bodo saltans</i>	9,545	1,936	4,345	1,000	2,498	1,300	1,438	2,400	4,238	3,450	5,346	1,200
5. <i>Oikomonas termo</i>	5,455	1,064	4,251	2,000	2,637	2,400	3,194	2,735	4,216	1,461	5,552	1,800
Ciliata												
6. <i>Didinium masutum</i>	500	500	800	800	500	60	50	89	63	90	190	400
7. <i>Balantidium coli</i>	5,840	900	450	456	936	967	400	973	960	980	1,000	810
8. <i>Paramecium caudatum</i>	2,165	1,113	4,147	3,472	2,469	7,404	1,818	6,547	7,316	7,279	13,076	993
9. <i>Vorticella</i>	5,350	849	634	734	687	938	851	1,314	919	637	1,396	888
10. <i>Podophrya fixa</i>	4,385	490	919	927	638	847	373	999	943	1,336	1,500	500
11. <i>Stentor coeruleus</i>	347	65	58	69	1,937	97	987	100	109	109	129	68
12. <i>Stylonchia pustulata</i>	3,438	315	918	1,216	1,346	2,139	949	3,418	1,130	1,000	240	328
13. <i>Spasmostoma</i>	4,385	417	806	904	1,847	1,960	857	2,919	1,973	3,968	2,349	413

S_1 - as in Table - 2

S_2 - as in Table - 2

December to February revealed that the winter months and short detention time might have been responsible. The studies on BOD removal by indicator species have been carried out in the past. The monocultures of *Oscillatoria* removed 75-87% (Gaur *et al.*, 1960), *Chlorella* and *Scenedesmus* removed 70-80% (Khan, 1962) and upto 90% BOD was removed by *Chlorococcum infusiorum*, *Ankistrodesmus falcatus* and *Scenedesmus quadricauda* (Patil, 1979) respectively. Hosetti *et al.* (1988) reported that the BOD reductions by the complex pond community was 79.4%, *Chlorella vulgaris* 83.3%, *Scenedesmus quadricauda* 80.8%, *Tolypothrix tenvis* 74.1%, *Lyngbya allorgei* 78.2%, *Nostoc punctiformae* 82.5%, *Escherichia coli* 55%,

Shigella sonnei 51.6%, *Staphylococcus aureus* 47.5%, *Bacillus subtilis* 50.6% and *Paramecium caudatum* 68.1% respectively. Regression analysis showed that there was a significant correlation between the percent removal of BOD and number of algae and protozoa in both the samples ($r = 0.61$ and 0.62 at $P = 0.05$ for algae and protozoa in sample S_1 respectively and $r = 0.37$ and 0.50 at $P = 0.05$ for algae and protozoa in sample S_2 respectively). In the present study, BOD removal capacity of the natural pond can be enhanced by managing the pond scientifically and by constructing the pond in larger size as well as by providing a retention time of 8 to 10 days, as experienced in a similar environment at Karnataka University Campus, Dharwad (Hosetti, 1987).

The reduction in catalase activity in natural waste stabilization pond indicates reduction in organic content and BOD in the pond system. The measurement of catalase is taken as an indirect indicator of organic strength and

microbial status of effluents. It is revealed that catalase decompose hydrogen peroxide (into oxygen and water) which is a toxic substance produced in the cells during oxidative metabolism (Hosetti and Frost, 1998).

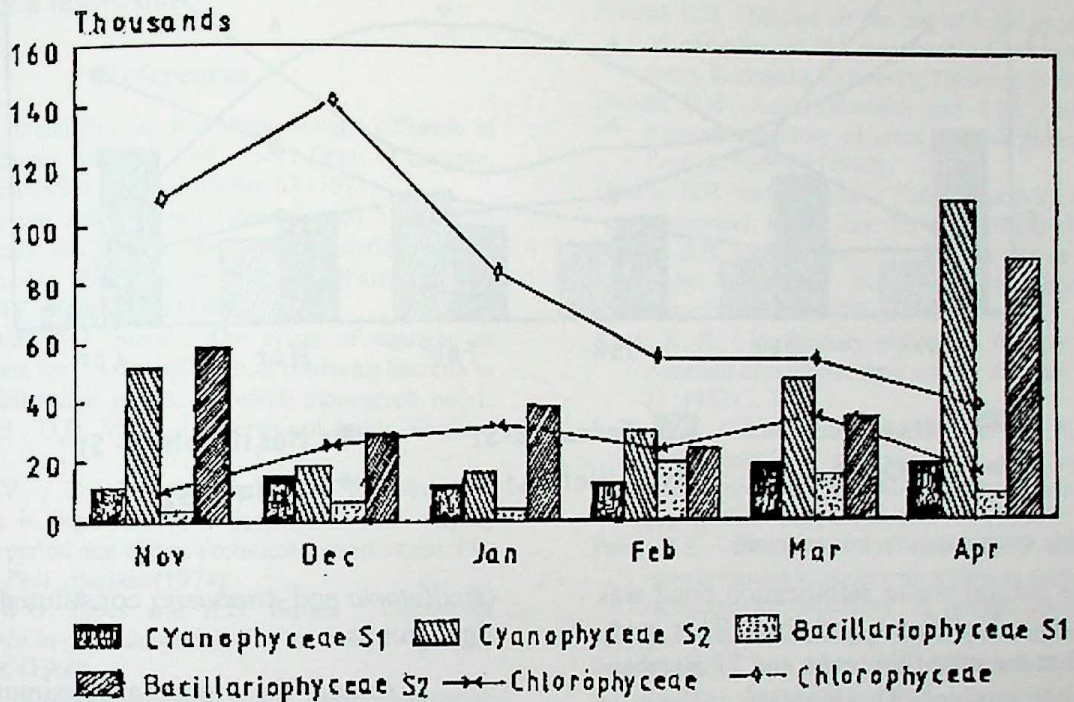


Fig. 1 : Monthly variations in Cyanophyceae Bacillariophyceae and Chlorophyceae members.

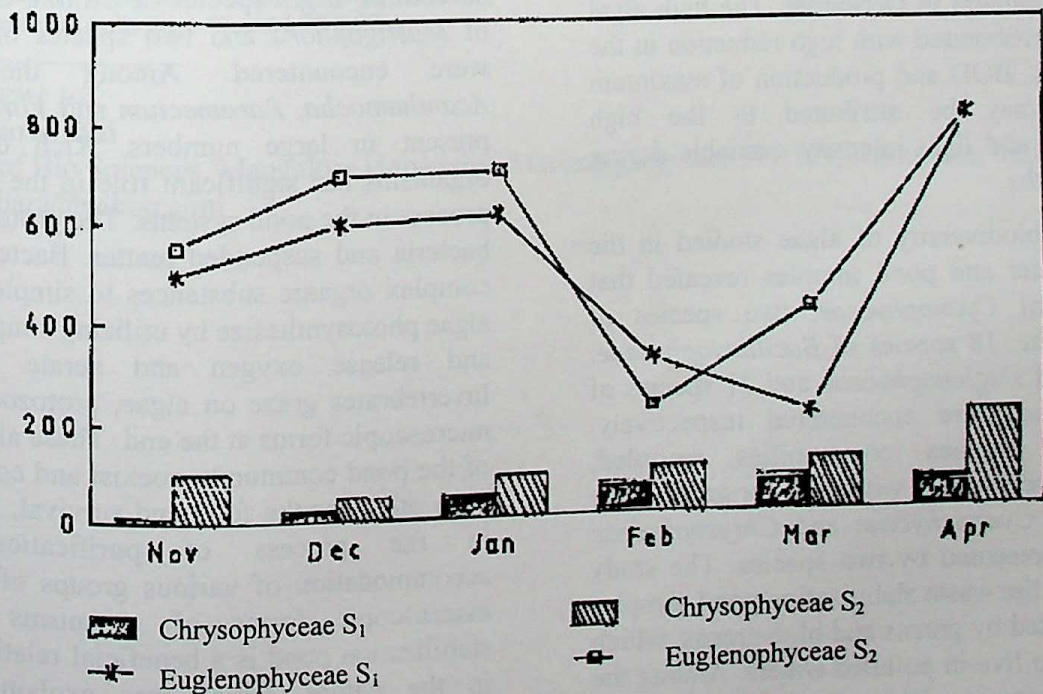


Fig. 2 : Monthly variations in Chrysophyceae and Euglenophyceae.

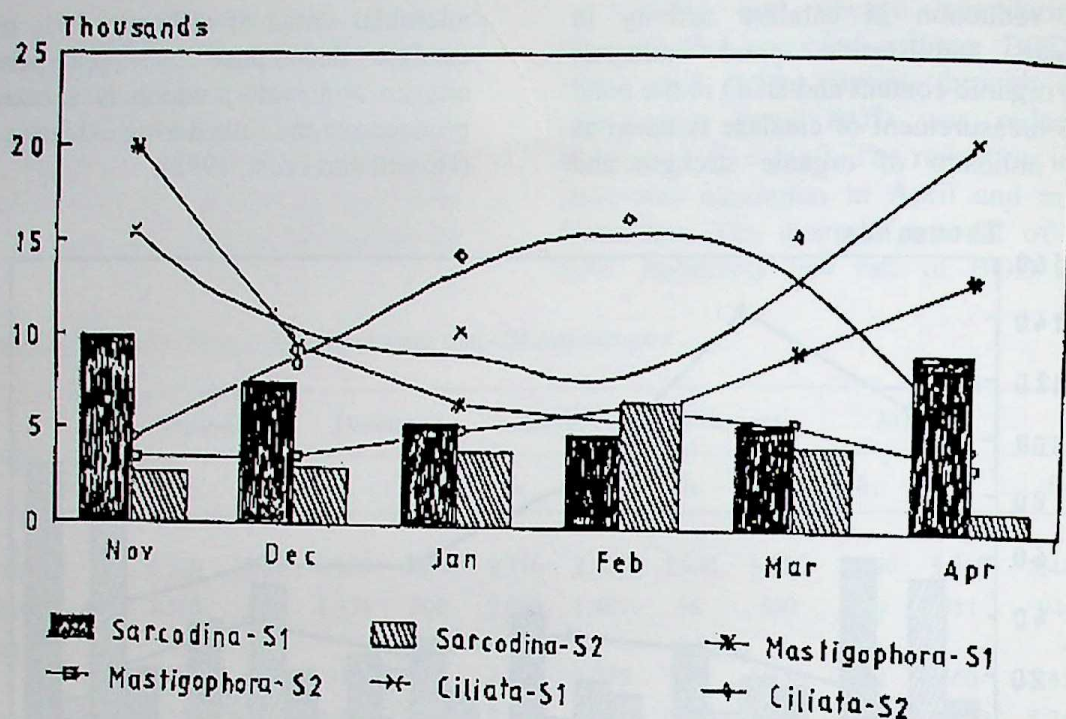


Fig. 3 : Monthly variations in Protozoan density.

The natural waste stabilization pond was rich in its microbial fauna and flora. This study revealed 13 members of protozoa and 71 members of algae from samples. The monthly analysis of algae density revealed that it was maximum in April and minimum in December. The high algal density is corroborated with high reduction in the levels of PO_4 , BOD and production of maximum DO. This may be attributed to the high temperatures and light intensity available during summer months.

The biodiversity of algae studied in the raw wastewater and pond samples revealed that 18 species of *Cyanophyceae*, two species of *Chrysophyceae*, 18 species of *Bacillariophyceae*, six species of *Euglenophyceae* and 21 species of *Chlorophyceae* were encountered respectively. Among the various communities recorded, maximum diversity was witnessed for green algae followed by *Cyanophyceae* and *Chrysophyceae* members represented by two species. The study revealed that the waste stabilization pond samples were dominated by greens and blue-greens, which are adapted to live in polluted waters. Among the various algal species, *Chlorella* and *Scenedesmus* were dominant representatives of green algae and

Oscillatoria and *Anabaena* constituted blue green algal groups.

Among the protozoa, maximum diversity was represented by *Ciliates* and minimum by *Sarcodina*. Eight species of *Ciliata*, three species of *Mastigophora* and two species of *Sarcodina* were encountered. Among the protozoa *Acanthamoeba*, *Paramecium* and *Vorticella* were present in large numbers. Rich diversity of organisms has significant role in the purification process in the pond systems. The protozoa feed on bacteria and suspended matter. Bacteria degrade complex organic substances to simpler products, algae photosynthesize by utilizing simple products and release oxygen and aerate the water. Invertebrates graze on algae, protozoa and other microscopic forms at the end. These all organisms of the pond community coexist and compete with each other for the food and survival, finally help in the process of purification. Hence, accommodation of various groups of micro and macroscopic forms of organisms in waste stabilization pond is a beneficial relation existing in the nature, which was explained by the scientists and employed in wastewater abatement technology.

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Toxicity of leaf extract of Yellow Oleander *Thevetia nerifolia* on Tilapia.

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Abstract : The usefulness of leaf extract as an ideal source of 'piscicide' in shrimp farming is described. Leaf toxins are safe, eco-friendly and biodegradable. The fish Tilapia, *Oreochromis mossambicus* was exposed to the extract and the percentage of mortality at the end of 24, 48, 72 and 96 hrs was recorded. The respective toxic range of aqueous, alcohol and acetone extracts of 24 hr LC_{50} and 96 hr LC_{50} values were found to be respectively 1118.79-330.30, 699.24-129.02 and 749.95-347.23 mg dry extract/litre for *Oreochromis mossambicus*. The LC_{50} values observed in different time periods in the fish exposed to aqueous extract were relatively high compared to the values obtained in acetone and alcohol extracts. Use of leaf extract, as piscicide in aquaculture farms is considered advantageous when viewed against the backdrop of using persistent chemicals.

Key words : Toxicity, Leaf extract, *Thevetia nerifolia*, Shrimp farming, Tilapia.

Introduction

Many chemicals with pesticidal properties are regularly synthesized and marketed every year for use against insects and other pests of aquaculture, household and storage, which are not safe, eco-friendly and biodegradable. There is widespread environmental degradation due to indiscriminate use of chemicals. In this backdrop, bioremediation in particular phytoremediation have gained importance. In the place of chemicals plant extracts, which play a vital role in the control of vectors, can be used. These are also inexpensive, readily available and have low toxicity. In shrimp farming also, during the preparation of the pond, chemicals are used for the eradication of predator organisms as fishes. In their place, it will be advantageous to use plant extracts. The present study has been undertaken to find out the suitability of the leaf extract of Yellow Oleander *Thevetia nerifolia* in shrimp farming. The test animal used here is *Tilapia Oreochromis mossambicus*.

Materials and Methods

Collection and maintenance of fish : Specimens of *Oreochromis mossambicus* were procured from the Fisheries Department (Government of Tamilnadu), Chetput, Chennai. They were stocked

and maintained in the laboratory for 10 days at $28 \pm 2^{\circ}\text{C}$ temperature. They were fed with commercially available pellet feed and the water was changed daily. Fish measuring 6-7cm in length and 5-6 g in weight were used for the present study. Feeding was stopped 2 days prior to the commencement of experiments in order to reduce the quantum of excretory products in the test water.

Preparation of leaf extracts : Leaves of Yellow Oleander *Thevetia nerifolia* were collected locally from Valasaravakkam, Chennai. The leaves were dried and made into powder. The leaf powder was mixed well with water, alcohol and acetone. The extract was obtained by filtering to know the exact amount of plant materials present in the extract; a known volume of extract was taken in a weighed beaker and kept open for drying. After drying, the beaker was again weighed and noted the amount of plant materials present in the extract.

The leaves of the plant were dried in a shadow place for about 15-20 days. The dried leaves were removed from the branches and powered in a mixer. 88 g of leaf powder was taken in one litre round bottom flask and 250 ml of acetone (solvent) was added and mixed thoroughly. The flask was tightly plugged with cotton bed and kept in refrigerator for a week. At

the end of the week, the solvent extract (stock solution) filtered through cotton bed was kept in the refrigerator in order to avoid evaporation. The other procedures followed were similar to that of aqueous extract for the solvent extract preparation. Likewise, the alcohol extract was also prepared.

Acute toxicity test : Static bioassays for determining acute toxicity were conducted as recommended by the U.S. Environmental Protection Agency (EPA, 1975). Test solutions were prepared as described in standard methods for the examination of water and wastewater, (APHA, 1960), from the stock solutions using tap water as diluent.

For assessing the toxicity of each of the selected concentration of the extracts, 10 animals were exposed to 5 litres of test solutions taken in a plastic bucket of 10-litre capacity. The test water was renewed every 24 hours (as described by the U.S. EPA, 1975) with least disturbance to the

animals, to maintain the dissolved oxygen content and plant extracts. Controls were also maintained. Survival was observed after 24, 48, 72 and 96 hours and the number of survivors at the end of each period was noted and the percentage of mortality calculated. Further, the acute toxicity test concentration and 95% fiducial limits were calculated by using Finney's Probit analysis with log "e" transformations, as described by Mohapatra and Rangarajan, (1995).

Results

The acute toxicity values (LC_{50} values) for *Oreochromis mossambicus* maintained in the extracts of *Thevetia nerifolia* found out at different time intervals are given in Tables 1-3 along with the 95% fiducial limits. The 24 hr LC_{50} and 96 hr LC_{50} in aqueous, alcohol and acetone were respectively 1118.79-330.30, 699.24-129.02 and 749.95-347.23 mg dry extract/ litre for *Oreochromis mossambicus*. While calculating

Table - 1 : Estimated LC_{50} values (24 hrs-96hrs) and 95% fiducial limits for *Oreochromis mossambicus* exposed to aqueous leaf extract of *Thevetia nerifolia*

Exposure period (hrs)	LC_{50} (mg/l)	95% Fiducial limits	
		Upper limit (mg/l)	Lower limit (mg/l)
24	1118.79		
48	566.80	1495.18	862.64
72	464.05	854.06	437.03
		620.17	333.61

Table - 2 : Estimated LC_{50} values (24 hrs-96hrs) and 95% fiducial limits for *Oreochromis mossambicus* exposed to alcohol leaf extract of *Thevetia nerifolia*.

Exposure period (hrs)	LC_{50} (mg/l)	95 % Fiducial limits	
		Upper limit (mg/l)	Lower limit (mg/l)
24	699.24		
48	523.22	915.96	539.15
72	304.90	678.58	399.41
96	129.02	445.86	210.61
		245.18	61.56

95% fiducial limits, an asymptote was reached during 96 hr at 330, 129 and 347 mg dry aqueous, alcohol and acetone extracts/litre in that order due to attainment of lethal threshold in the extract concentration. Similar lethal threshold was also

noticed at 120 hr in the air breathing fish *Anabas testudineus* treated with 0.288 mg lindane /litre, 6.60 mg disyston /litre and 0.91 mg furadan /litre (Bakthavathsalam and Srinivasa Reddy, 1982). Further, the lethal threshold of the facultative air-

breather *Colisa lalia* at 120 hr for lindane was found to be 0.135 mg/ litre (Ramalingam and Srinivasa Reddy, 1982). But in *Lepidocephalichthys thermalis* exposed to

carbofuran, the same was attained only at 72 hr (Bakthavathsalam, 1986). Similar 96 hr median lethal concentration (g/l) using four leaf extracts was obtained after exposure of *Oreochromis*

Table - 3 : Estimated LC₅₀ values (24 hrs-96hrs) and 95% fiducial limits for *Oreochromis mossambicus* exposed to acetone leaf extract of *Thevetia nerifolia*

Exposure period (hrs)	LC ₅₀ (mg/l)	95 % Fiducial limits	
		Upper limit (mg/l)	Lower limit (mg/l)
24	749.95	880.07	632.70
48	550.04	651.97	464.05
72	411.58	539.15	368.71
96	347.23	437.03	278.66

niloticus and *Cyprinus carpio*, *B. blasnifera*, *V. negunda*, *A. indica* and *T. rumphii* (Cagauan, 1995). Generally, the time of attainment of equilibrium (lethal threshold) reached varied from one toxicant to another and from one animal to another.

The LC₅₀ values (1118.79 and 330 mg dry extract/litre respectively at 24 hr and 96 hr) observed at different time periods in fish exposed to aqueous extract (Table 1) were relatively higher when compared to similar values (749.95 and 347.23, 699.24 and 129.02 mg dry extract/litre respectively at 24 hr and 96 hr) obtained in acetone and alcohol extracts (Tables 2 & 3). The difference observed in the LC₅₀ values of aqueous extract during initial hours (1-15 hr) was relatively very high when compared to later hours (24-96 hr). The difference observed in the present study between 1 and 15 hr LC₅₀ values of aqueous extract to *Oreochromis mossambicus* was noteworthy as far as the toxicities of aqueous, alcohol and acetone extracts are concerned.

Fish affected by the toxicity of *Thevetia nerifolia* behaved abnormally. Such abnormal behaviors include sluggishness, loss of equilibrium, gasping for air on the water surface and finally lack of response to outside stimuli. The behavioral response may be the initial reaction to exposure of toxic substances when compared to uncontaminated natural environment.

The present study reveals that the leaf extract contains sufficient quantity of toxins, which can kill fishes. Therefore this could be advantageously used in aquaculture operation especially shrimp culture. The leaf of this plant contains an untapped resource available abundantly hence may be used as an eco-friendly piscicide alternative to chemical pesticides.

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Health monitoring of farm labourers engaged in MIPC 50 WP field sprays.

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Abstract : Investigations were undertaken to monitor the health status of farm labourers engaged in field sprays of MIPC 50 WP (Hexamycin, Mipcin), a carbamate insecticide on cotton crop, as per the protocol approved by the Central Insecticide Board. The insecticide sprays (0.1 %) were undertaken for six hr on three consecutive days on 1.2 hectares of cotton crop per day, using Aspee napsak sprayers. The spray personnel (mixers, loaders and sprayers) with protective clothing did not reveal any alteration in clinical, hematological and blood bio-chemical profile during exposure and post exposure periods. The spray personnel without protective clothing showed only a marginal reduction in their blood cholinesterase activity during the exposure period.

Key words : Health monitoring, Farm laborers, Insecticide sprays, MIPC, Mipcin, Carbamate insecticide.

Introduction

The use of chemicals in industry, agriculture, public health and households has immensely contributed for the welfare of mankind. However, their indiscriminate and/or uncontrolled applications and usage have also resulted in pollution of the environment. Pesticides, more preciously the insecticides, are one of the most widely used chemicals. These chemicals are of much value as crop and grain protectants in agriculture, for treatment of parasitic infestation in livestock and for the control of insect vectors in public health.

The use of environmentally persistent organochlorine insecticides like DDT has been banned in many countries and their use is restricted in several others. As a result, the other categories such as organic phosphate, carbamate and pyrethroid insecticides are increasingly used. However, such insecticides are not environmentally persistent, their extensive application in agriculture continues to pollute the soil and water and as such the environment. Exposure to such harmful pollutants can cause subtle adverse effects in man and animals (Chauhan, 1988). Further insecticide sprays in crop fields are usually undertaken by the farmers without following the prescribed essential precautions. As a result, the farm labourers are

exposed to such harmful chemicals. Studies pertaining to health monitoring of farm workers engaged in pesticide sprays are meagre.

MIPC (2-isopropylphenyl N-methyl carbamate, isoprocab, mipcin) is anew carbamate insecticide extensively used for the control of sucking pests of cotton, plant hoppers of rice and aphids on safflower (Murthy *et al.*, 1990; Haung and Pang, 1992; Yang Limei *et al.*, 1995). The literature on toxicity of MIPC is inadequate. There are no reports on the health records of personnel involved in its field spray operations. Accordingly, the Pesticide Registration Committee, Govt. of India made it mandatory to generate detailed health record data of men and livestock exposed to pesticide sprays in crop fields. Therefore, the present study was undertaken to evaluate the adverse effects, if any, among farm workers exposed to MIPC sprays in crop fields.

Materials and Methods

A detailed 'protocol' for monitoring of health status of men and livestock exposed to carbamate insecticide field sprays in crop fields was formulated and approved by the Central Insecticides Board, Govt. of India, Faridabad. The test sample of the insecticide formulation (Hexamycin 50% wettable powder) of MIPC, as

supplied by M/s Bharat Pulverising Mills Limited, Mumbai was used for the study.

Twenty-four clinically healthy volunteers, who had no illness or previous exposure to pesticides during the 30 days preceding the trial, were selected. They were randomly allotted to two groups, each containing 12 persons. The Group I personnel were of normal clothing i.e. as usually wore by the farmers during spray operations and the Group II personnel were

provided with protective clothing consisting of face mask, goggles, rubber apron, rexine cap, rubber hand gloves and gum boots. Table 1 shows the distribution of persons as per the nature of work allotted to the two groups. A total area of 10 hectares of cotton crop field was selected for spray operation; out of which 7.2 hector, was marked for MIPC 50 WP spray and the rest for blank

Table - 1 : Group-wise distribution of personnel engaged for MIPC (50 WP) field spray operations.

Nature of work	Number of personnel engaged	
	Group -I (Normal clothing)	Group -II (Protective clothing)
MIPC field spray	6	6
MIPC mixing/loading	3	3
MIPC total	9	9
Blank field spray	2	2
Blank mixer/loader	1	1
Blank total	3	3
Grand total	12	12

Table - 2 : Schedule of MIPC (50 WP) sprays on cotton field by personnel with normal clothing and protective clothing.

Sr. No.	Particulars	Days of spray operation		
		First	Second	Third
1	Area of cotton field covered (ha)	1.2	1.2	1.2
2	Spray volume utilized (L)	600	600	600
3	MIPC concentration in spray liquid (% a.i)	0.1	0.1	0.1
4	Quantity of a.i utilized (kg)	0.6	0.6	0.6
5	Quantity of w.p. utilized (kg)	1.2	1.2	1.2
6	Duration of spray	6	6	6
7	Number of spraymen	6	6	6

formulation spray. The details of spray schedules by the two groups are given in Table 2. The MIPC/blank formulation sprays were carried out using Aspee napsak sprayers (16 L. capacity).

All the volunteers were accommodated at a convenient place during the trial period, where they were provided with adequate lodging and boarding facilities. They were constantly under

the supervision of two Medical Officers. All the personnel were subjected to detailed physical/clinical examination as per the 'protocol' daily during the trial period. Blood samples were obtained from each one of them on three pre-exposure and exposure days and subsequently on third and seventh day doing post-exposure period, for hematological (Archer and Jeffcott, 1977)

estimations (cell counts, hemoglobin and ESR). The blood samples were also subjected to the following biochemical analyses :

- Red blood cell and plasma cholinesterase (Metcalf, 1951)
- Blood plasma urea nitrogen (diacetyl monoxime method)
- Serum total protein (Biuret method)

- Serum albumin (BCG dye binding method)
- Serum glutamic oxaloacetate transaminase (SGOT) and glutamic pyruvate transaminase (SGPT) (Reitman and Frankel method) and
- Serum alkaline phosphate (SAP) (Kind and King's method).

Table - 3 : Biochemical values of personnel exposed to MIPC (50 WP) field spray with normal clothing and protective clothing.

Parameter	Normal clothing personnel						Protective clothing personnel					
	Blank spray			MIPC 50 WP spray			Blank spray			MIPC 50 WP spray		
	Pre-exposure	Exposure	Post-exposure	Pre-exposure	Exposure	Post-exposure	Pre-exposure	Exposure	Post-exposure	Pre-exposure	Exposure	Post-exposure
Whole blood cholinesterase (μ m of Ach hydrolysed/ml)	0.979 [0.026]	0.990 [0.017]	0.983 [0.021]	0.953 [0.024]	0.875 [0.020]	0.949 [0.017]	1.000 [0.023]	0.996 [0.021]	0.987 [0.009]	0.996 [0.019]	0.983 [0.020]	1.000 [0.017]
Red blood cell cholinesterase (μ m of Ach hydrolysed/ml)	0.774 [0.018]	0.788 [0.014]	0.779 [0.017]	0.748 [0.018]	0.681 [0.018]	0.747 [0.015]	0.798 [0.009]	0.796 [0.010]	0.790 [0.011]	0.799 [0.011]	0.776 [0.010]	0.802 [0.011]
Plasma cholinesterase (μ m of Ach hydrolysed/ml)	0.205 [0.016]	0.202 [0.018]	0.204 [0.014]	0.205 [0.009]	0.194 [0.030]	0.203 [0.006]	0.204 [0.017]	0.197 [0.016]	0.197 [0.007]	0.199 [0.012]	0.202 [0.012]	0.199 [0.008]
Serum total protein (g/dl)	5.790 [0.496]	5.696 [0.586]	5.790 [0.485]	6.626 [0.410]	6.246 [0.400]	6.690 [0.380]	6.036 [0.746]	5.976 [0.726]	6.115 [0.780]	6.330 [0.326]	6.213 [0.296]	6.375 [0.315]
Serum albumin (g/dl)	2.663 [0.233]	2.636 [0.253]	2.500 [0.240]	2.693 [0.106]	2.740 [0.103]	2.705 [0.105]	2.523 [0.283]	2.506 [0.240]	2.495 [0.285]	2.490 [0.086]	2.500 [0.080]	2.490 [0.850]
Blood plasma urea nitrogen (mg/dl)	29.166 [5.063]	29.043 [5.333]	28.335 [5.160]	29.980 [3.556]	29.113 [3.383]	31.780 [3.250]	31.703 [6.616]	32.050 [6.503]	32.075 [7.255]	33.816 [2.993]	35.170 [2.993]	34.475 [2.980]
SGOT (units/ml)	30.553 [4.440]	30.553 [3.450]	34.830 [4.195]	31.220 [5.653]	28.700 [5.120]	30.105 [4.030]	41.330 [16.600]	41.553 [15.953]	37.995 [14.195]	42.403 [7.753]	45.476 [7.800]	41.105 [8.135]
SGPT (units/ml)	17.886 [5.096]	20.330 [3.530]	19.500 [4.130]	21.183 [2.010]	23.036 [2.090]	22.885 [2.470]	19.110 [5.290]	19.330 [5.383]	18.165 [5.105]	17.366 [3.806]	17.216 [3.816]	18.275 [4.855]
SAP (KA units/ml)	20.010 [4.083]	20.506 [3.583]	20.345 [4.240]	24.596 [3.966]	24.863 [3.863]	24.470 [4.355]	30.173 [6.843]	30.020 [6.013]	31.285 [7.905]	31.110 [4.590]	31.150 [4.250]	31.105 [4.505]

Figures in parenthesis indicate S.E.

The blank values means of three persons

The MIPC values are means of nine persons

All the biochemical analyses, except cholinesterases, were carried out by the respective methods as described by Nath (1976). The results of biochemical and hematological analyses are

presented as combined means of three pre-exposure, three exposure and two post-exposure observations of personnel of blank and MIPC sprays in both the groups. The data were

statistically analysed by using 't' test (Sheth *et al.*, 1972).

Results and Discussion

Clinical examination : The reports of clinical examination as per the protocol by the Medical Officers did not indicate any detectable adverse reaction, sign or change in behaviour among all the spray personnel in normal clothing or protective clothing groups.

The data of biochemical profile of spray personnel in both protective clothing and normal clothing groups (Table 3) did not indicating statistically significant changes in any of the observations made. However, there was marginal reduction in the cholinesterase activities among the normal clothing personnel during exposure to the

Table - 4 : Hematological values of personnel exposed to MIPC (50 WP) field spray with normal clothing and protective clothing.

Parameter	Normal clothing personnel						Protective clothing personnel					
	Blank spray			MIPC 50 WP spray			Blank spray			MIPC 50 WP spray		
	Pre-exposure	Exposure	Post-exposure	Pre-exposure	Exposure	Post-exposure	Pre-exposure	Exposure	Post-exposure	Pre-exposure	Exposure	Post-exposure
Total red blood cell (million/cu mm)	5.35 [0.35]	5.31 [0.27]	5.29 [0.41]	5.58 [0.41]	5.59 [0.57]	5.53 [0.41]	5.94 [0.51]	5.83 [0.59]	5.83 [0.65]	5.38 [0.42]	5.44 [0.38]	5.49 [0.43]
Total white blood cell (thousands/cu mm)	6.76 [0.61]	7.17 [0.38]	6.75 [0.50]	6.31 [0.33]	6.69 [0.31]	6.61 [0.33]	6.55 [0.88]	6.06 [0.90]	6.17 [0.90]	6.67 [0.59]	6.76 [0.60]	6.67 [0.60]
Hemoglobin (g%)	15.07 [0.75]	15.00 [0.79]	15.06 [0.78]	13.08 [0.52]	13.07 [0.55]	13.00 [0.54]	12.59 [0.40]	12.64 [0.50]	12.3 [0.60]	12.11 [0.39]	12.08 [0.42]	12.14 [0.40]
Erythrocyte sedimentation	4.22 [0.63]	4.22 [0.40]	4.66 [0.86]	3.88 [0.45]	4.07 [0.45]	3.49 [0.37]	4.99 [0.79]	5.33 [0.43]	4.83 [0.74]	4.70 [0.39]	4.47 [0.46]	4.77 [0.41]
Neutrophil count (%)	59.88 [2.87]	59.88 [1.74]	59.16 [1.44]	63.51 [1.71]	62.55 [1.62]	60.94 [1.09]	62.22 [2.82]	62.99 [2.45]	63.16 [3.12]	59.58 [1.50]	60.29 [1.30]	59.83 [1.25]
Lymphocyte count (%)	34.55 [2.77]	33.11 [1.16]	35.83 [2.02]	31.11 [1.65]	31.14 [1.68]	33.44 [2.00]	30.11 [2.81]	29.10 [3.09]	27.99 [2.66]	33.81 [1.41]	32.55 [1.55]	33.16 [0.91]
Eosinophil count (%)	2.10 [0.33]	2.99 [0.68]	3.49 [0.40]	2.18 [0.28]	2.62 [0.40]	2.55 [0.25]	3.66 [0.27]	3.77 [0.48]	4.66 [0.27]	2.40 [0.37]	3.14 [0.29]	3.49 [0.36]
Monocyte count (%)	2.77 [0.54]	3.21 [0.57]	2.66 [0.27]	2.66 [0.40]	2.84 [0.30]	2.44 [0.31]	3.55 [0.51]	3.66 [0.48]	4.16 [0.40]	3.58 [0.49]	3.47 [0.31]	3.44 [0.40]

Figures in parenthesis indicate S.E.

The blank values means of three persons

The MIPC values are means of nine persons

insecticides sprays (91.0 to 94.6%) as compared to their pre-exposure cholinesterase activity (considered as 100 %). Subsequently, during the post-exposure period their cholinesterase activities returned to near normal levels. The reduction in cholinesterase activity, though

marginal, indicated a slight degree of absorption of MIPC through contact or inhalation during the spray operations.

The hematological data of the spray personnel with normal clothing and protective clothing also did not suggest statistically variable

values during pre-exposure, exposure and post-exposure periods of the MIPC spray schedules (Table 4).

From the observations of the present study, it may be concluded that the exposure to 0.1 percent MIPC field sprays @ 6 h per day may not pose a health risk to the farm workers. However, it is advised that the spray personnel should wear appropriate protective gear to avoid absorption of the insecticide.

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Short Communication

Antimicrobial activities of *Eusteralis deccanensis* and *E. quadrifolia* essential oils.

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Abstract : Antimicrobial activity of the essential oils of *Eusteralis deccanensis* and *E. quadrifolia* were investigated on *Bacillus subtilis*, *B. megaterium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Rhizopus oryzae*, *Aspergillus niger* and *Colletotrichum musae*. Both the oils possess growth inhibitory activity against most of the microorganisms tested.

Key words : Antimicrobial activity, *Eusteralis deccanensis*, *E. quadrifolia*.

Introduction

The genus *Eusteralis* Raf. (Lamiaceae) is a tropical aromatic herb, *E. deccanensis* Panigrahi, a herbaceous weed, found to be growing wild, near streams and shallow waters of South India, whereas *E. quadrifolia* (Benth.)

Panigrahi is very common in the rocky slopes and hills, growing in the crevices (Thoppil and Jose, 1995). There is little report on the medicinal properties of *Eusteralis*. In the present study, these two plants have been investigated for their inhibitory activity against bacteria and fungi.

Table - 1 : Antimicrobial properties of *Eusteralis deccanensis* and *E. quadrifolia*.

Microorganisms	Essential oils and dilutions in acetone **						Standards
	<i>E. deccanensis</i>			<i>E. quadrifolia</i>			
	1:0	1:1	1:2	1:0	1:1	1:2	
Bacteria							Gentamycin sulphate (40 mg/ml)
<i>Bacillus subtilis</i>	26	24	20	28	20	18	48
<i>B. megaterium</i>	28	25	24	25	22	20	45
<i>Escherichia coli</i>	16	16	16	40	33	28	29
<i>Pseudomonas aeruginosa</i>	16	16	16	16	16	16	40
<i>Staphylococcus aureus</i>	25	22	19	24	21	19	35
Fungi							Nystatin (50 IU)
<i>Rhizopus oryzae</i>	30	25	20	30	25	20	31
<i>Aspergillus niger</i>	24	20	18	30	23	20	38
<i>Colletotrichum musae</i>	16	16	16	18	16	16	30

* Including the diameter of the filter paper disk

+ Mean value of three independent experiments

Materials and Methods

E. deccanensis and *E. quadrifolia* growing wild in the Western Ghats were collected during October-December and authenticated at the

Herbarium of Botany Department, University of Calicut, where voucher specimens were deposited (CALI 51045 and CALI 36347 respectively). Shade dried aerial plant parts were hydrodistilled

in a Clevenger apparatus at 100°C for 4 h. The aromatic essential oils obtained were used for investigation.

To test the antimicrobial activity, five bacteria and three fungi (origin: MTCC Gene Bank, Institute of Microbial Technology, Chandigarh, India) were used. Antimicrobial activity was studied using the filter paper disk diffusion method (Benson, 1990). The degree of growth inhibition was evaluated after 48 h. and compared with the growth inhibition results obtained from the controls (Gentamycin for bacteria and Nystatin for fungi).

Results and Discussion

The results are recorded in Table 1. The essential oil of *E. quadrifolia* was more active

than the standard gentamycin against *E. coli*. Both the essential oils showed moderate inhibitory activity against most of the microorganisms tested. The essential oils of *Eusteralis*, because of their strong microbicidal property and superiority over commercial bactericide, may prove effective as a herbal protectant against a wide spectrum of pathogenic bacteria and fungi, since herbal microbicides are non-toxic, biodegradable and eco-friendly.

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Review paper

Fungal toxicity with special reference to mycotoxins.

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Abstract : Mycotoxins are fungal secondary metabolites formed by consecutive series of enzyme-catalysed reactions from a few biochemically simple intermediates of primary metabolism. These mycotoxins can enter the human and animal food chain by direct or indirect contamination. Mycotoxins are equally harmful to animal and human beings. Realizing the importance of mycotoxins to the health of man and animals there have been concentrated efforts to develop highly sensitive analytical methods for detection and proper determination of mycotoxins in food, mixed feeds and feed ingredients, animal tissue, blood, urine and milk.

Most of the mycotoxins are identified and most current research on it is concentrated on increasing sensitivity accuracy and reproducibility and above all to decrease the time of determination. A detailed review of mycotoxin and their detection is summarised in the paper.

Key words : Mycotoxins, Epidemiological data, Storage system.

Introduction

What we eat actually depends on what we get and not what we want. Approximately 20% food becomes unsafe before reaching the consumers. Moulds are known to produce a wide variety of biologically active compounds on Agricultural and other consumable commodities. Human poisoning by fungal metabolites caused due to consumption of contaminated food stuffs has been a matter of serious concern in different parts of the World. The deleterious effects and its consequences of eating poisonous mushrooms are known since the dawn of civilization but it was only in the 18th Century when the people could realize the effect of certain filamentous fungi (moulds) as serious health hazards to human beings. Such fungi may be saprophytic or pathogenic.

Fungi produce a vast and bewildering array of toxic metabolites, which abound in organic environments (Turner, 1971). The magnitude of their effect depends upon the nature of the fungus and the situation under which it interacts with other organisms. Toxins play a

pivotal role in determining inter and intra species relationships among the fungi. Some of the fungal toxins, that are mostly secondary metabolites, are highly toxic to the vertebrates and are termed as mycotoxins.

Mycotoxins and mycotoxins producing fungi : Mycotoxins are produced by a wide range of fungi, but the main producers belong to three important genera viz. *Aspergillus*, *Fusarium* and *Penicillium*. Some of the important mycotoxins producing fungi and their toxic metabolites implicated in the outbreak of mycotoxicosis are in Table 1.

Mycotoxins elaborated by the species of *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* are most wide spread in our food items and some of these do not get destroyed even at a fairly high temperature of 260°C or so. These four fungal genera are capable of producing about three dozen toxins depending upon the substrate and genetic makeup of the strain. Alternariol and tenuazonic acids are the important mycotoxins elaborated by *Alternaria alternata*. Many other species like *Pithomyces charatarum*, *Stachybotrys atra*,

Rhizoctonia leguminicola etc. also produce metabolites harmful for human and animal health.

History

The first clearly recognized episode-involving mycotoxin in human toxicosis was that of ergot poisoning through the relationship between ergotism and causal fungus (*Claviceps purpurea*), which infected the rye ears, was not conclusively established till the 18th century. The ergot alkaloids are derivatives of lysergic acid. Toxicologically these compounds are oxytoxics i.e.

they stimulate the smooth muscles of the uterus. They are also weak vasoconstrictors. The last outbreak of ergot poisoning is described by Gabbai *et al.* (1951) in France where about 200 people got ill due to consumption of molded rye which was used as flour and as bakery product.

Authentic reports about mould produced toxins in Cattle (1924), Stachybotryotoxicosis in horse (1931) *Fusarium* toxicity in Swine (1936). Mouldy corn poisoning in Horses (1940) started appearing in scientific literature since the early part of the present Century. Maximum concern

Table – 1 : Various toxins produced by fungi and their biological effects.

Toxins	Producing fungi	Biological effects
1. Aflatoxins	<i>Aspergillus flavus</i>	Hepatotoxic
	<i>A. parasiticus</i>	Carcinogen
2. Ochratoxin	<i>A. ochraceus</i>	Nephrotoxin
3. Citrinin	<i>Penicillium viridicatum</i>	Nephrotoxic
	<i>P. citrinum</i>	
4. Patulin	<i>P. patulum</i>	Death of cattle
5. Trichothecene	<i>Fusarium tricinctum</i>	Dermal necrosis
	<i>F. solani</i>	Hemorrhage
	<i>F. graminearum</i>	Vulvovaginitis
6. Zearalenone	<i>F. roseum</i>	Abortion

Table – 2 : Natural occurrence of mycotoxins in food.

Mycotoxin	Commodity	References
Aflatoxin	Maize	Krishnamachari <i>et al.</i> 1975
		Mishra, 1977
	Paddy	Bilgrami <i>et al.</i> , 1981; Mall <i>et al.</i> 1983
		Sreenivasamurthy, 1965
		Nusrath and Ravi, 1983
	Wheat	Mishra, 1977
	Sorghum	Bhadraiah and Ramarao, 1983
	Groundnut	Sreenivasmurthy <i>et al.</i> , 1965
	Cotton	Mehan <i>et al.</i> , 1991
	Dry fruits	Singh Anjana, 1983
Zearalenone	Spices	Singh Anjana, 1983
Ochratoxin	Maize	Bilgrami <i>et al.</i> , 1980
	Paddy	Nusrath and Ravi, 1983
	Dry fruits and spices	Singh, Anjana, 1983.
Citrinin	Paddy	Nusrath and Ravi, 1983
	Dry fruits and spices	Singh, Anjana, 1983
	Dry fruits and spices	Singh, Anjana, 1983

Table - 3 : Mycotoxicoses associated with acute primary disease of live starlet of poultry (Pier *et al.* 1980).

Mycotoxicosis	Animal species	Primary syndrome
1. Aflatoxicosis	Poultry, swine, cattle, dog	Acute hepatitis, hemorrhagic
2. Ergotism	Cattle, sheep, chicken	Gangrenous, necrosis; nervous seizures; reproductive failure
3. Facial eczema	Sheep, cattle	Cholangio hepatitis; photosensitivity
4. Fusariotoxicoes		
(a) verruotoxicosis	Swine	Emesis
(b) t-2 toxicosis	Swine, cattle, poultry	Dermonecrosis; gastroenteritis
5. Diacetoxyscirpenol	Swine	Oral, gastroenteric necrosis hemorrhages
6. Leukoencephalomalacia	Horses	Nervous depression, inco-ordination
F-2 toxicosis (zearalenone)	Swine	Estrogenism
Ochratoxicosis	Swine, turkeys	Nephropathy
Paspalum staggers	Cattle, sheep, horse	Ataxia
Slaframine toxicosis	Cattle, sheep	Salivation, diarrhoea, polyurea
Stachybotryotoxicosis	Horses	Dermonecrosis, gastroenteritis, haematopoietic depression
Tremorgen intoxication	Cattle, sheep, dog	Fasciculation; atoxia; prostration.

Table - 4 : Mycotoxicosis in which Analytic and/or epidemiologic data suggest human involvement (Hayes, 1980).

Disease	Species	Substrate	Etiologic agent
1. Alimentary toxic aleukic (ATA) (on septic angina)	Man	Cereal grains and toxic bread	<i>Fusarium sporotrichoides</i>
2. Dendrocladiotoxicosis	Horse	Fodder (skin contact/inhaled)	<i>Dendrocladium toxicum</i>
3. Kashin beek disease 'urov disease'	Man	fodder particles	<i>Fusarium sporotrichiella</i>
4. Stachybotryotoxicosis	Man	Cereal grains	
	Man	Hay, cereal grains fodder	
	Horse	(skin contact inhaled hay dust)	<i>Stachybotrys atra</i>
	Other livestock		
5. Cardiac beriberi	Man	Rice	<i>Fusarium species</i>
6. Ergot	Man	Rye, cereal grain	<i>Claviceps purpurea</i>
7. Balkan encephalopathy	Man	Cereal grains	<i>Penicillium species</i>
8. Rey's syndrome	Man	Cereal grains	<i>Aspergillus species</i>
		Groundnuts	
9. Hepatocarcinoma	Man	Cereal grains groundnuts	<i>Aspergillus species</i>
10. Pink rot	Man	Celery	<i>Sclerotinia species</i>
11. Onychia	Man	Millet	<i>Phoma sorghina</i>

was however felt in the post Second World War when in Russia a disease outbreak in human being, which was named as Alimentary toxic aleukia (ATA). This disease was first recorded in eastern Siberia in 1913 but its deleterious effects were not fully realized at that time. Russians eating mouldy (due to infestation of *Fusarium*/*Cladosporium*) over wintered and snow covered grains, suffered with several dermal necrosis, leucopenia, hemorrhage rashes and destruction of bone marrow. In acute cases nasal, gastric and intestinal hemorrhages may also occur and necrotic

lesions are caused in throat, on the lips and on skin of the nose, jaws and the eyes. Subsequently some interesting observations were made and it was found that over wintered sample of cereals retained their toxicity even after seven years of mould infestation. The culture filtrates of *Fusarium* and *Cladosporium* grown at low temperature were more toxic than those maintained at room temperature. Highest toxicity was associated with materials collected at the time of maximum spore production (Joffe, 1983). In certain areas, mortality was upto 60%.

Historically the year 1960 is very important because of the scientific concern generated about the mycotoxins. During this year, about one lakh beautiful game birds-Turkey perished in England within a short span of time without leaving any trace about the cause of death. This mysterious disease greatly baffled the veterinary scientists who were unable to find out the causes or cure of disease. Since it was difficult to give any scientific name to the disease it was designated as "Turkey -X-disease". It was however soon realized that the sudden doom of turkey pouts

was due to consumption of meal served to them. Systematic analysis of the meals was therefore, initiated but it was found to be free of bacteria, viruses or any other known toxic chemicals. The affected birds expressed loss of appetite; letharginess, weakening of the wings and drying of legs etc. It was found that the meal served to the 'turkey birds' was prepared of peanuts that were actually imported from Brazil by a ship named Rossette. The meal was thus nick-named as "Rossettee meal".

Table - 5 : Basic Steps in chemical analysis of mycotoxins (after Romer, 1976).

Step	Description	Purpose
1. Sampling	Probe of automatic sampler	Representative sample
2. Sample preparation	Grinding, mixing, sub sampling	Representative sample
3. Extraction	Shaker or blender	Separate the toxins from compounds insoluble in the extraction solution.
4. Clean up	Liquid-liquid partitioning (separatory funnel) Column-chromatography Divalent metal clean up (Pb+2, Fe++ , Cu+2)	Separate the toxin from groups of compounds in the sample extract.
5. Final separation	Thin layer chromatography (TLC) Gas-liquid chromatography (GLC) Liquid-liquid chromatography (LC) Mini column chromatography Fluorescence on TLC Plate	Separate the toxin from remaining compounds in the sample extracts that might inter-fare with the toxins.
6. Detection and quantitation	Fluorescence in solution U.V. Absorption in solution GLC-flame detector	Detection and measurement of response
7. Confirmation	TLC-separation and detection of derivative of mycotoxins biological test mass spectrometry	Identification of chemical compound

A vigorous search by British microbiologists concluded that the meal that had killed the birds was heavily infested by fungus *Aspergillus flavus* link ex fries. This fungus is very well distributed in nature and its conidia are wide spread in air and soil. Unable to chemically characterise the nature of toxin, they named the toxic factor as AFLATOXINS (Sergeant *et al.* 1961).

Several mycotoxins have been reported on cereals, pulses, oil seeds, dry fruits, spices, green fruits, vegetables, dried fish and shrimps, milk and milk products, different types of meat as well as

wide variety of other consumables articles. Most of these toxic substances find their way to human system through different routes. These harmful chemicals are in one food chain but unfortunately their long-range effects have not been properly evaluated.

Mycetismus versus mycotoxicosis : Toxicity of fungi to man and animals can arise by mycetismus (mushroom poisoning) or by mycotoxicoses (Emmons *et al.*, 1977). Mycetismus occurs by the ingestion of certain mushroom type fungi which can cause a toxic response as a result of (a) protoplasmic poisoning (*Amanita phalloides*, A.

verne; *A. tennifolia* and *Galerina venenata* (b) neurologic effects (*Amanita pantherina* and species of *Inocybe*, *Clitocybe*, *Lepiota* and *Hebeloma*); and (c) gastro intestinal irritants (*Lectarius terminosis*, *Tricholoma pardinum* and species of *Agaricus*, *Boletus*, *Clavaria*, *Russula* and *Clitocybe*).

In contrast to mycetismus, mycotoxicosis is poisoning by ingestion of toxins of fungal origin in foods that have been altered as damaged by the growth of toxin producing mould fungi. Mycotoxins are in general low molecular weight; non-antigenic fungal metabolites capable of eliciting a toxic response in man and animals. Mycotoxins are known to produce illicit effect on variety of food commodities including agricultural crops. An acquisition of it by the host is by ingestion, inhalation or contract and quite small amounts of the compounds can represent significant health hazards (WHO, 1979; Hayes, 1980). At high concentration many mycotoxins can produce acute disease syndrome while at lower levels they can be carcinogenic, mutagenic, teratogenic or estrogenic and may reduce the growth rate of young animals and can even interfere with native mechanisms of resistance and impair immunologic responsiveness making the animal more susceptible to infections (Pier *et al.* 1980).

The diversity of symptoms of mycotoxicoses association differs with the different fungal species. The main genera of fungi associated with mycotoxins include species of *Aspergillus*, *Penicillium*, *Fusarium*, *Claviceps*, *Alternaria*, *Pithomyces*, *Stachybotrys*, *Phoma* and *Diplodia*.

Mycotoxins are fungal secondary metabolites formed by consecutive series of enzyme catalysed reactions from a few biochemically simple intermediates of primary metabolism, e.g. acetate mevalonate, malonate and certain amino acids (Turner, 1971, Steyn 1980). The main biosynthetic reactions include condensation, oxidation/reduction, alkylations and halogenations steps that create a remarkable range

of secondary compounds. The principal pathways involved in the formation of mycotoxins are viz., the polyketide route (e. g. aflatoxins), the terpene route, (e.g. trichothecene) the amino acids route (e.g. gliotoxin) and the tricarboxylic acid route (e.g. rubrotoxin). Some mycotoxins (e.g. cyclopiazonic acid) are formed from a combination of two or more of the principal pathways (Turner 1971)

Source of mycotoxins : Mycotoxins can enter the human and animal food chain by direct or indirect contamination. In direct contamination the food material support the toxigenic mould growth. Possible route for mycotoxin contamination of human and animal food may be as (adopted from Jarvis 1976) : -

Mould damaged foodstuffs

- (i) Agricultural produce
e.g. Cereals, oil seeds (groundnut) fruits, vegetables
- (ii) Consumer foods (Secondary infections)
compounded animal feeds (secondary infections).
- (iii) Residue in animal tissue and animal products
e.g. Milk dairy produce, meat
- (iv) Mould- ripened foods
e.g. Cheese, fermented meat products, oriental fermentations.
- (v) Fermented Products
e.g. microbial proteins, enzymes, food additives, e. g. -vitamins.

Almost all foods will be susceptible to mould growth at some stage during their production processing, transport and storage. In indirect contamination the food ingredient is contaminated with mycotoxins.

Mycotoxins in agricultural crop : Cereals can be highly susceptible to fungal growth when still in the field, during processing or during storage (Stoloff, 1976). Small grains (wheat, sorghum, oats, rye, barley and rice) unless abused in storage or after preparation appears to be less susceptible to mycotoxin formation, than the larger grains

such as Maize. The main mycotoxins that have been detected in cereals are the aflatoxins, sterigmatocystin, ochratoxin-A, zearalenone, T-2 toxin and vomitoxin (Scott, 1973; Stoloff 1976; Bennett and Shotwell 1979; Pathre and Mirocha, 1979; WHO, 1979; Anon., 1980; Vensonder and Hesselstine 1981).

The incidence of occurrence varies with climatic conditions prevailing at the time of harvest, transportation and during later storage conditions. During harvesting of the grains moisture plays an important role to subsequent mould colonization which may further lead to production of mycotoxins by secondary metabolites, for e.g. Maize, which is harvested at a high moisture level and thus it, is more susceptible to mycotoxin producing fungi. A list of mycotoxins in agricultural products is given in Table 2.

Realizing the importance of seed health, since last few decades a lot of work has been done on the seed mycoflora of various agricultural commodities including cereals, pulses, millets, oil seeds as well as in spices and dry fruits. Seed mycoflora of medicinal plants as well as of food and feed were also reported by various workers (Bilgrami *et al.*, 1980; Chandra *et al.*, 1981; Srivastava and Gupta, 1981; Vijaya 1982; Esteves, 1984; Augustine *et al.* 1984; Abate and Abegaz, 1985; Basak and Mirdha, 1985; Mislivec, 1977; Hegde and Hiremath, 1987; Shrivastava and Gupta 1984; Hasheem, 1990; Frisvad and Samson, 1991; Kanchanlata, 1991; Kumar and Roy, 1993; Weidenborner and Kunz, 1993; Roy and Kumar, 1993; Shah *et al.*, 1993; Hendry and Cole, 1993; Dodan *et al.*, 1994; Kholbe *et al.*, 1994; Peter *et al.*, 1994; Ranganathaiah, 1994; Rajkumari D. *et al.*, 1993; Nelson *et al.*, 1994; Sinha, 1994).

Mycotoxins in animal products : Mycotoxin contaminated feeds fed to certain type of animals, e.g. aflatoxin to dairy cattle, ochratoxin to pigs, can pose a potential or real threat because of the possible carry over of toxic residues into the animal products and thus into the food chain of human being. Mycotoxicoses associated with acute

primary disease of live starlet of poultry is given in Table 3.

Chemical properties of mycotoxins : Mycotoxins comprise of a group of chemically unrelated fungal metabolites. These may be polypeptide, alkaloid, benzoquinone, anthraquinone, xanthone, coumarin, terpene and their various derivatives.

Aflatoxins are group of highly oxygenated heterocyclic bisfurocoumarin metabolites. The major members of this group are designated as B1, B2 and G1 and G2. The aflatoxin fluoresces strongly in ultra-violet light (Ca, 365 nm). B1 and B2 produce blue fluorescence where as G1 and G2 produce green fluorescence. B2 and G2 are dihydro derivatives of parent compounds B1 and G1.

Ochratoxins are a group of seven isocoumarin amides of 1- phenyl aniline, among which ochratoxin A is the most toxic and it is a dihydro-isocoumarin carboxylic compound. Citrinin is a yellow crystalline chemical compound having p- quinone methide structure. The presence of hydrogen bonding between the carboxyl group and both orthohydroxyl groups in citrinin molecule has also been confirmed by x-ray diffraction. Zearalenone is an enone derivative of 6-B-resorcylic acid lactone.

Detection of mycotoxins in natural products : Most of the main mycotoxins can now readily be identified quantitatively and qualitatively and most current research is concentrated on increasing sensitivity, accuracy and reproducibility and above all, to decrease the time of determination (Romer, 1976; Pohland *et al.*, 1979). Accurate and reliable analytical methods are the corner-stone for a sound understanding of all aspects of mycotoxicology. The major steps involved in mycotoxin determination are given in Table 5.

The time between sampling and analysis is critical and samples held in environmental conditions conducive to mould growths can easily develop mycotoxins within 1-2 days. Among the analytical techniques generally used HPLC (High

performance liquid chromatography) is most widely accepted.

Control : There is no doubt that the exclusion of mycotoxins from human and animal food chain would result in significant avoidance of human illness, reduction in productivity, loss of food or feed, livestock mortality, reduced growth rate and feed efficiency in live stock, livestock infertility and reduced market price for commodities (FAO/WHO/UNEP Conference on Mycotoxins, 1977).

The basic strategies i. e. prevention, inactivation and detoxification have been adopted to control the adverse effect of mycotoxins. However low temperature storage of grains in treatment of grains with propionic acid and it's sodium salt during storage, radiation and UV light treatment, treatment with aqueous plant extracts (specially with neem) has been proved to be responsible for reducing/slowing down the infestation and growth of mycotoxin producing fungi and subsequently production of mycotoxins.

Recent advances : Several workers all over the world have investigated the problem of mycotoxin in different food commodities. The world health organization (WHO) has fixed certain limits of mycotoxins in different food and feed stuffs. In India Indian scientists to mycotoxin problem have showed considerable awareness. The problem of mycotoxicosis has been reported in different parts of country. The fungal seeds responsible for mycotoxicosis are available in air and they deteriorate the seed content and produce toxins too especially during storage. In Rajasthan people normally use alternative foods for their survival during famine and they stored them in traditional storage systems. These foods were found toxicated during a study and produce toxins. (Bohra.1998). In groundnut both biotic factors such as soil insects and soil pathogens and abiotic factors such as end of season drought and soil temperature during crop growth can lead to aflatoxins contamination (The Hindu, 2002). The propagation of plants is accomplished by seeds and there are evidences that these microbes may be

systematically transferred from one generation to another through seeds (Mycock *et al.*, 1992; Campbell *et al.*, 1995).

Study of aflatoxin structure revealed the relationship of toxicity with the presence of a double bond on the furan ring of aflatoxins. Aflatoxin B₁ and G₁ have this double bond and they are carcinogenic whereas B₂ and G₂ lack the bond and are only weak toxic. These aflatoxins are absorbed from the gut and then pass to liver where they become highly reactive. The epoxide bridge formed at the double bond on furan ring enables the toxin to bind to DNA and depurinate it, causing genomic lesions that can lead to heptomas.

Conclusion

Although knowledge of fungal toxins has advanced considerably in the recent past, the fungal toxins have created scare due to their carcinogenic and mutagenic effects. Future progress in this fascinating area of research to great extent will depend upon a collective approach of scientists drawn from diverse disciplines like biochemistry, medicine and plant pathology. Relatively little attention has been paid towards control of mycotoxins. Education of farmers to improve the method of storage, surveillance of food stuffs and animal feeds for the presence of mycotoxins and application of appropriate food processing technology to separate the contaminated food components from the clean ones.

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Fungal toxicity with special reference to mycotoxins.

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Impact of urbanization on Bellandur Lake, Bangalore-A case study.

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Abstract : Lake and river water is the prime source for drinking, irrigation and other domestic purposes. Bellandur Lake is one of the major Lakes of Bangalore city. The addition of effluents from urbanized Bangalore city has changed the characteristics of the Lake from being a natural ecologically healthy Lake to an artificial reservoir of domestic sewage and industrial effluents. The DO of the Bellandur Lake water ranged from 3.8-6.3 mg/l. The Bellandur lake water BOD ranged from 89-99 mg/l due to absorption of pollutants by aquatic flora in lake system. If the present state of affairs continues for long, the Bellandur Lake may soon become an ecologically inactive Lake.

Key words : DO, BOD, COD, Lake.

Introduction

Lakes and rivers are the prime sources of drinking, irrigation and other domestic purposes. About 80% of the earth's surface is covered by water yet the inland fresh water availability in account for less than one percent. Park (1997) opined that the Lakes contribute globally 0.088% to freshwater resource, which is generally available for drinking and domestic purposes. Today many natural water bodies in India receive millions of litres of wastewater and agricultural runoff, with different concentrations of pollutants in varying forms. Bangalore city is one of the largest cities in India, and popularly known as Garden city, had 262 Lakes in 1982. Bellandur Lake is one of the major Lakes of Bangalore city and is subjected to severe pollution stress from the urban community.

Materials and Methods

Study area : Bellandur Lake is located near the village Bellandur adjacent to Bangalore-HAL road towards southeast of Bangalore city. Bellandur Lake covers an area of 920 acres and lies between longitude of 77°39'15" W- 77°46'00" E and 12°55'59" S- 12°57'20" N of latitude. Bellandur tank has a catchment area of about 3.5 Km². It is a rain fed tank, but since a few years, it is being used as a disposal site for municipal and industrial wastes. The drainage system is dendritic and

centripetal type. During the monsoon, the overflow water from Bellandur Lake reaches Varthur Lake.

Selection of sampling sites

Sample 1 is located at the Challaghatta Kere valley Drain that flows from Vasant Nagar and Jaya Mahal areas, through Jogi Palya, Indira Nagar, Domlur, HAL Area and finally joins Bellandur Lake. Waste Water Samples from these drains were collected during February 2000. The sampling technique used was composite sampling for a period of seven days. The average values for the seven-day period are presented.

Sample 2 is located at Chinnagara Kere valley drain flows from Cubbon Pet, through Sampangiramnagar, Richmond Town, Shantinagar, Neelasandra, Rajendranagar, Koramangala, Chinnagara and joins Bellandur Lake. The sample was collected near Koramangala drain.

Sample 3 Agara Kere valley drain flows from Jaya Nagar, NIMHANS, Bairasandra, John Nagar, Madiwala, Venkatapura and Agara and joins the Chinnagara Drain. The sample was collected from the Chinnagara drain.

Sample 4 The inlet of the Bellandur Lake near HAL. The wastewater was collected near the outlet of STP (inlet for Bellandur Lake).

Sample 5 represents sample collected near outlet of the Bellandur Lake that leads to Varthur Lake.

Sample 6 and 7 located before and after the treatment facility for the wastewater from K&C Valley catchment in the BWSSB Wastewater Treatment Plant.

Wastewater and water samples were collected in jerry cans during June and samples for DO and BOD were brought in separate glass containers in icebox. The samples were analysed in the laboratory using Standard Methods (APHA, 1995).

For Annual projection of pollution load, the calculation involved is one of conversion of per day value into per month and per year as follows.

Flow: The litres per day flow are converted into m^3/sec . and then to kg/sec as follows :

$$\text{Flow MLD} \times 1000 = \text{m}^3/\text{day}.$$

$$\frac{\text{m}^3/\text{day}}{24 \times 60 \times 60} = \text{m}^3/\text{sec}.$$

$$\frac{\text{Pollution load mg/l}}{1000} = \text{g/l}$$

$$\text{Pollution load g/l} \times \text{Flow m}^3/\text{sec} = \text{g/sec}.$$

$$\frac{\text{g/sec}}{1000} = \text{kg/sec}$$

(MLD = Million Liters per Day)

Pollution load litre is cancelled with m^3 . The obtained kg/sec value is multiplied with $24 \times 60 \times 60$ that gives kg/days . This on multiplication with 30 & 365 gives kg/month and kg/year , respectively.

Table-1 : Qualitative analysis of the three major drains and Bellandur Tank.

Parameters	Challaghatta Kere valley	Chinnagara Kere valley	Agara Kere valley	Inlet near HAL	Varthur outlet
Color	Black	Light brownish	Black	Light brownish	Light greenish
Temperature ($^{\circ}\text{C}$)	27.5	27	26.5	23	23.3
pH	7.2	8.2	7.5	7.83	7.67
Electrical conductivity	2750	2691	2598	2272	2120
Alkalinity	585	585	460	568	478
Chlorides	112.1	106	108	194	198
Nitrates	330	340	360	323	225
TS	610	690	750	632	584
TSS	110	100	125	165	158
TDS	600	590	625	467	426
Settleable solids	8	6	7	12	2
DO	Nil	Nil	Nil	3.8	6.3
BOD	340	322	330	39	32
COD	1040	1020	980	99	89
BOD/COD ratio	0.32	0.31	0.33	0.39	0.35

*All values in mg/l , except pH, EC.

Results and Discussion

Extremes of pH hinder the survival of living organisms. The balance in an ecosystem is maintained when pH is between 5.5 and 8.5. The pH of inflow sewage was found to be within the permissible limit of 5.5-9.0 according to BIS standards.

Color of the Bellandur Lake water varied from light greenish to green, because of algal

blooms. The temperature of the Bellandur Lake water ranged from $23-23.3^{\circ}\text{C}$ (Table 1). The temperature of the influent and effluent were found to be within the permissible limits.

The alkalinity ranged from 478-568 mg/l . A number of bases, viz. carbonates, bicarbonates, hydroxides, phosphates, nitrates, silicates, borates etc., contribute to alkalinity (Garg, 1998). The Challaghatta Kere valley drain and Chinnagara

Kere valley drain recorded the highest of 585 mg/l. The sample from Agara Kere valley showed 460 mg/l. The total alkalinity ranged from 470-519 mg/l at inflow point and 414- 463 mg/l in the

Sewage Treatment Plant. The percentage removal of alkalinity is only 11.7% by secondary sewage treatment. This is because only organic matter decomposes during the treatment. The alkalinity is

Table-2 : Data of the samples analyzed before and after secondary treatment of sewage.

Flow : 75.94 MLD			
Parameters	Inlet to sewage treatment plant	Outlet to sewage treatment plant	Percentage removal
pH	7.5	7.75	-
Temp, °C	23.5	22.5	-
Color	Blackish	Light blackish	-
Alkalinity	497.16	438.5	11.7
Chlorides	112.5	95.8	14.8
Nitrates	328.83	219	43.41
Total solids	731.81	311.6	53.6
TSS	186.4	70.8	38
TDS	496.6	185	38
Settleable solids	7.6	1.45	80.9
DO	0.25	2.8	-
BOD	339	35.6	90
COD	1037.2	100.8	90
BOD/COD ratio	0.322	.35	-

*All values in mg/l, except pH.

contributed by a number of bases like carbonates, bicarbonates, hydroxides, phosphates, nitrates, silicates, borates etc. added to the water ecosystem through disposal of treated and untreated sewage, leading to increase in nutrient quantity. The alkalinity let into Bellandur Lake every year is estimated as 6076.95 kg/year.

The presence of chlorides in the wastewater streams is due to industrial sector using organic and inorganic chloride compounds, besides domestic sewage. Chlorides recorded highest value of 112.1 mg/l at Challaghatta Kere valley sample whereas Chinnagara Kere valley and Agara Kere valley recorded 106 and 108 mg/l respectively. The presence of chloride concentration in a water source is used as an indicator of organic pollution by domestic sewage (NEERI, 1979). The amount estimated that 2655.3 kg/year chlorides are added to Bellandur Lake after the treatment. This seems to be enormously high. The average removal of chlorides in the secondary treatment is about 14.8%.

Nitrate is the oxidized form of nitrogen and in water, it's most important source is biological oxidation of nitrogenous organic matter of both autochthonous and allochthonous origin which include domestic sewage, agricultural runoff and effluents from industries (Saxena, 1998). The sample from Agara Kere valley contained 360 mg/l nitrate, the highest among the three streams, probably because of hospital discharges. Nitrate concentration depends on the source of water and nature of the catchment of a water body (Sahai and Sinha, 1969; Senayya and Zafar, 1979). The removal of nitrate in the secondary treatment is up to 43.41 %, because of nitrate utilization by the bacteria in aeration tank. The quantity of nitrate estimated is 6070.03 Kg/year.

The highest TDS recorded in Agara Kere sample is 625 mg/l, and samples from Challaghatta Kere valley and Chinnagara Kere valley showed 600 and 590 mg/l respectively. The Agara Kere valley sample contained a highest of 125 mg/l total suspended solids and samples from

Challaghatta Kere valley and Chinnagara Kere valley had 110 and 100 mg/l respectively, the permissible limit being 100 mg/l. Verma (1978) observed a large amount of dissolved solids in their study area of Kedarabad drain. An estimated 5127.64 kg/year of TDS get into Bellandur Lake, causing eutrophication. The disposal of both suspended and dissolved solids leads to sedimentation (Saxena, 1998). The settleable

solids include inorganic and undissolved solids, which settle to the bottom of the ponds causing siltation of Lakes. The removal of total solids is (TDS + TSS) 25- 53% in the course of treatment. The removal of TDS ranged from 24-54%. The maximum permissible limit for TDS is 2000 mg/l according to BIS standards. The TDS at outflow of STP ranged from 106-382 mg/l, well within the permissible limits.

Table-3 : Average quality of pollution estimated. Average flow : 75.94 MLD.

Parameters	Inflow			Outflow		
	Kg/day	Kg/month	Kg/year	Kg/day	Kg/month	Kg/year
Alkalinity	37.752	1132.58	13779.7757	16.6491	499.4753	6076.95
Chlorides	8.5429	256.2872	3118.1614	7.2747	218.2428	2655.2876
Nitrates	24.8564	745.6933	894.8304	16.6301	498.9058	6070.0209
TS	55.5729	1667.1883	20284.125	23.6619	709.8587	8636.6142
TSS	14.1546	424.6394	5166.447	6.1171	183.5136	2232.7488
TDS	37.7102	1131.3088	13764.2574	14.0483	421.4501	5127.6432
Settleable solids	0.5771	17.3136	210.6491	0.1101	3.3032	40.1896
BOD	25.7426	772.2788	9396.0597	2.7033	81.1006	986.7248
COD	78.7618	2362.8544	28748.0627	7.6544	229.6333	2793.8726

The Bellandur Lake water estimated to be 12 ml/l of TSS at the discharge site and is still less near outfall, because of the aquatic flora and weeds filters the solids before reaching the outfall. The removal of TSS in the STP is 62% and it depended on the nature of organic matter. On an average, the Total Solids disposed into the Lake from STP is estimated to be 8636.61 kg/year whereas the quantity of suspended solids gaining entry is 2232.75kg/yr.

The DO of Bellandur Lake water ranged from 3.8-6.3 mg/l. The lowest value is observed at discharge point where mixing of effluents occurs immediately. It has increased near outfall due to photosynthetic activity. The DO in the sewage is nil in all the samples, indicating its septic nature. In the Challaghatta Kere Sample BOD was 340 mg/l. The BOD of the Lake water ranged from 225-323 mg/l, with higher values near the discharge point. The Bellandur Lake showed low BOD, ranged from 89-99 mg/l due to absorption by aquatic flora. The increased DO is due to the aeration process and removal of organic matter in

the treatment. The DO is nil in the influent, whereas it increased up to 3 mg/l in the lake. The useful aerobic bacteria flourish and bring about aerobic biological decomposition of wastewater, which will continue to absorb oxygen for many months, and it is not practically feasible to determine this ultimate oxygen demand (Garg, 1998). The BOD of the influent is more than 300 mg/l and after treatment, it is found to be nearly within the permissible limits but some time exceeded the permissible limits of 30 mg/l. The removal of BOD is up to 92%. The amount of BOD disposed is estimated to be 986.8 Kg/year. More BOD means more oxygen requirement to degrade organic matter. The biodegradable and non-biodegradable organics entering the lake have been estimated to be 2793.9 Kg/year. The non-biodegradable organics contribute to accumulation of chemical constituents, and eventually the recovery of the Lake may become irreversible.

The maximum permissible limit for COD for discharge of effluents into surface waters is 250 mg/l according to BIS standards. However, all

the samples showed COD values above the permissible limit. The Challaghatta Kere and Chinnagara Kere samples showed a highest value of 1040 and 1020 mg/l respectively. The COD determines the chemically oxidisable organic matter present in sewage (Garg, 1998). The COD incoming into STP ranged from 951-1017 mg/l. This on an average is removed up to 90%. The outflow STP ranged from 79-142 mg/l and the values are therefore within the permissible limit of 250 mg/l according to BIS standards. If the ratio is between 0.92-1.0, the wastewater can be considered virtually biodegradable and above 0.63, considered quite amenable to biological treatment. All the samples showed values below 0.63; hence appear to contain some non-biodegradable organics.

Bellandur Lake is an ecologically important tank, which feeds Varthur and other tanks besides recharging groundwater table in the K and C Valley. It remains dry during summer and refills during monsoon. However, of late, due to continuous flow of municipal and industrial effluents from urbanized Bangalore city, the lake is overflowing throughout the year, thus changing the characteristics of the lake from being a natural ecologically healthy lake to artificial reservoir of

sewage and industrial wastes. With such substantial quantities of pollutants released into the lake, the biotic survival becomes difficult. The algal blooms appear throughout the lake almost round the year. If the present state of affairs continues, situation may arise that Bellandur Lake might become an ecologically inactive lake.

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Determination of a limiting nutrient regulating algal biomass using *in situ* experiments of Nutrient Enrichment Bioassay (NEB) and empirical relations of nutrients and chlorophyll-a.

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Abstract : Long-term nutrient contents and nutrient ratios indicated that phosphorus was a potential limiting element for algal growth. *In situ* experiments of nutrient enrichment bioassay supported the evidence of P-limitation. However, regression analyses of \log_{10} -transformed chlorophyll-a (CHL) against TP (R^2 values < 0.25) showed that seasonal CHL was not closely related to flux of phosphorus during all seasons. Also, two dimensional graphical approach of Trophic State Index (TSI) showed that most values of TSI (CHL) -TSI (TP) and TSI (CHL) -TSI (SD) were less than zero, indicating factors other than phosphorus limited algal biomass (CHL -TP < 0), and that non-algal particles dominated light attenuation (CHL -SD < 0). The weak empirical relations and trophic deviations were explained well by the experiment of NEB-II that was conducted during a period of high inorganic turbidity. Overall results suggest that phosphorus is the primary element regulating the system productivity, but the system also were highly influenced by rapid flushing and high inorganic turbidity.

Key words : Nutrient enrichment bioassay, Trophic state, Seasonal variation, TSI.

Introduction

For several decades, nutrient enrichments have been considered undesirable, because it impairs human use of water for purposes such as water supply and recreation. In man-made lakes, eutrophication can increase water treatment costs (Ryding and Rast, 1989), contribute to taste and odor problems (Walker, 1985), and increase production of trihalomethane during a treatment for drinking waters (Palmstrom *et al.*, 1988). It also reduces water transparency and increases algae and aquatic macrophyte growth, resulting in a limitation in recreational use (Cooke *et al.*, 1993).

In general, phosphorus (P) and nitrogen (N) are frequently considered the major nutrients controlling the eutrophication. Phosphorus, because of loss from the water column by sedimentation and lack of a gas phase, is often considered the most important limiting nutrient, which thereby regulates eutrophication processes of temperate, inland waters (Vollenweider, 1968; Schindler, 1974; Jones and Bachmann, 1976;

OECD, 1982; Vrba *et al.*, 1995). Nitrogen limitation also occurs, especially in middle to low latitudes (White, 1982; Vincent *et al.*, 1984; Jones *et al.*, 1989).

Recent studies of man-made lakes, however, demonstrated that factors controlling lake productivity are not so simple due to their large variations in morpho-hydrodynamic characteristics (Thornton, 1990; Wetzel, 1990). Rapid flushing (water residence time < 60 -100) often resulted in losses of phytoplankton from the water column (OECD, 1982; Hoyer and Jones, 1983; Soballe and Bachmann, 1984; Lind *et al.*, 1993) and significant modifications in the empirical relations of algal biomass, measured as chlorophyll-a concentrations, and nutrients in N-or P-limiting systems (Hoyer and Knowlton and Jones, 1996). Under this situation, nitrogen or phosphorus may not be a useful parameter for diagnosis of the eutrophication (Smith, 1983) but hydrodynamic conditions may be a primary factor controlling the lake processes (Soballe and Kimmel, 1987). Therefore, identification of key factors regulating the lake productivity has an important implication

for efficient lake management. The objective of the study is to identify limiting factors using *in situ* experiments of Nutrient Enrichment Bioassay (NEB) and evaluate site specific relations among trophic parameters.

Materials and Methods

The descriptions of the study site and data base : This study was carried out in Uiam Reservoir, which is located in the northern part of the Korean peninsula. The reservoir was constructed in 1967 on the upper part of Han-River and has the watershed area of 7,709 Km², the storage capacity of 80 million m³, and short annual water residence time of 5-17 days.

Annual total precipitation is 1200 mm in the watershed and one third of the total rainfall occurs mainly during summer monsoon period of July -August. The volume of inflow coming to the reservoir depends on the discharge from two sources of Chuncheon dam in the northern Han-River and Soyang dam in the southern Han-River and annual, thus hydrodynamic characteristics of the reservoir are directly influenced by the two dams. Three sampling sites were chosen for the study; site 1 (S1) is near the dam of Uiam Reservoir, while site 2 (S2) and site 3 (S3) are located in the middle of the reservoir and are influenced by discharges from the Cheonchon dam and Soyang dam, respectively. These sampling sites were monitored during 1993-2000 by the Ministry of Environment, Korea. Parameters monitored are conductivity, total phosphorus (TP), total nitrogen (TN), nitrate-N (NO₃-N), ammonia-N (NH₃-N), total suspended solids (TSS), Secchi depth (SD), and chlorophyll-a (CHL). Daily precipitation were used the data measured from the meteorological station near the dams.

In situ experiments of Nutrient Enrichment Bioassay (NEB) : Experiments of NEB were conducted near the S1 during May 11-17, 2002 (NEB-1) and S2 during June 16-22, 2002 (NEB-2) to determine which nutrient control the reservoir productivity or algal growth. Epilimnetic water samples were collected from the S1 and S3 and

was mixed in a 100 L polyethylene-lined container and dispensed into 4 L translucent polyethylene cubitainers. Unfiltered surface water was suspended at the half Secchi depth and incubated 6 days in the cubitainers. The experimental conditions are the control (C, no additions), P (15 µg L⁻¹ as phosphorus), NH₄-N (0.05 mg L⁻¹ as ammonia-nitrogen), NO₃-N (0.50 mg L⁻¹ as nitrate-nitrogen), P+NH₄-N (15 µg L⁻¹ as phosphorus plus 0.05 mg L⁻¹ as ammonia-nitrogen), 2P (30 µg L⁻¹ as phosphorus), treatments. Phosphorus stock solution (Hach chemical Co.) was used to spike phosphorus treatments; and potassium nitrate (KNO₃) and ammonia chloride (NH₄Cl) stock solutions were used as sources of NO₃-N and NH₄-N, respectively. Nutrients (TN, TP, NH₄-N, and NO₃-N), turbidity, and non-volatile suspended solids (NVSS) were measured by standard method of APHA (1985), and chlorophyll-a (CHL) concentration was measured by using a spectrophotometer (Bechman Model DU-65) after extraction in hot ethanol (Sartory and Grobbelaar, 1984). In these experiments, the response of phytoplankton to the treatments was determined by measuring chlorophyll (CHL). The results were expressed as the ratio of final to initial CHL (CHL_f : CHL_i) or ratios of final CHL (CHL_f) in each treatment to CHL in the control. Differences among treatments were analyzed by one-way ANOVA Duncan's multiple range tests (SAS, 1991).

Data analysis : The study was hypothesized that patterns of water quality in the site of S2 may differ from those of S3 due to a difference in the hydraulic influence from two different dams. Based on the supposition, spatial and temporal trend were analyzed in the three sites using the data set of eight year. The seasons were discerned into three periods of premonsoon (January-June), monsoon (July-August), and postmonsoon (September-December) for the temporal analysis. The approaches of single and multiple regression analysis were used to evaluate effect of the precipitation on the water quality and the relations with water quality parameters. Evaluations of

Trophic State Index (TSI) using trophic parameters of CHL, TP, and Secchi depth followed the approach of Carlson (1977, 1991).

Results and Discussion

In situ experiments of NEB-I and NEB-II :
During the period of the NEB-I experiment, lake was calm (Secchi depth = 3.3m) and there was no significant inflow or rainfall. Non-volatile suspended solids (NVSS) averaged 1.3 mg L^{-1} , indicating that light availability was enough for algal growth. In the NEB-I, ratios of $\text{CHL}_i : \text{CHL}_f$ ranged from 0.85 to 2.38 depending on the

treatments. The ratios in the treatments enriched with phosphorus (> 1.80) were significantly ($p < 0.05$) greater than those of the control (C) or N treatments enriched with ammonia-nitrogen ($\text{NH}_4\text{-N}$) and nitrate-nitrogen ($\text{NO}_3\text{-N}$, Fig 1A). The ratios of $\text{CHL}_i : \text{CHL}_f$ in the P and $\text{P}+\text{NH}_4\text{-N}$ treatments did not differ from one another but was significantly ($p < 0.05$) greater than in the C (control) and N treatments (Fig 1A). Also, the ratios in the P treatment were $> 30\%$ greater than in the treatment of 2P, indicating that algal response is greater in the higher phosphorus concentrations. In the mean time, response in the

Table - 1 : The means and ranges of water quality parameters, based on the monthly data during 1993-2000.

Parameters	Mean \pm S.D.	Median	Range	n
TN (mg L^{-1})	1.602 ± 0.722	1.629	0.31 - 4.171	287
TP ($\mu\text{g L}^{-1}$)	59 ± 124	33	2 - 604	287
Secchi depth (m)	2.18 ± 0.84	2	0.5 - 4.7	283
Chlorophyll-a ($\mu\text{g L}^{-1}$)	10.4 ± 9.6	7.1	1 - 68.7	287
Conductivity ($\mu\text{S cm}^{-1}$)	77.5 ± 19.4	75	9.8 - 150	260
$\text{NO}_3\text{-N}$ (mg L^{-1})	1.225 ± 0.445	1.248	0.588 - 3.333	195
$\text{NH}_4\text{-N}$ (mg L^{-1})	0.135 ± 0.103	0.102	0.018 - 0.641	195

treatment of $\text{NO}_3\text{-N}$ was significantly ($p < 0.05$) less than the control (Fig 1A). The depressed response to in the treatment of $\text{NO}_3\text{-N}$ may be due to high ambient nitrate concentrations (nitrate-N $> 1000 \mu\text{g L}^{-1}$) regardless of seasons and year, but also due to inhibitory effect in high additions of potassium in the spike with nitrate-N.

In the NEB-II experiments, algal response showed distinct difference compared to the experimental results of the NEB-I. During the NEB-II experiment, NVSS values averaged 22 mg L^{-1} and intense rainfall occurred during the period. Thus, mineral turbidity ranged from 28 to 5 NTU, implying that underwater light penetration was significantly reduced during the experimental period. Ratios of $\text{CHL}_i : \text{CHL}_f$ in the control (C) and all treatments showed marked reductions compared to the results of NEB-II (Fig 1A, B). Algal response in the P treatment did not show a significant difference with the control, while it was significantly greater than that of the $\text{NH}_4\text{-N}$ and

$\text{NO}_3\text{-N}$ treatments (Fig 1B). Also, the ratios in the treatments of $\text{P}+\text{NH}_4\text{-N}$ and 2P were slightly greater and the response in the $\text{NH}_4\text{-N}$ (0.72) and $\text{NO}_3\text{-N}$ treatments (0.65) was less than the control (0.77). As shown in Fig 1B, initial mean absolute concentration of CHL in the ambient water was $18.3 \mu\text{g L}^{-1}$, but CHL values in the control showed distinct decreases after 6-day incubation. Also, mean CHL concentration in the initial condition did not significantly differ from the P treatment (Fig 1B). These results indicate that algal response in the NEB-II experiments was reduced due to rapid light attenuation in the water column by high inorganic solids. Thus, there was no discernable response in the P treatment in the P-limited system. Overall experiments of the NEB-II suggest that high inflow or rainfall may result in algal depression effect in the system.

Ambient nutrient contents : The averages, median means, and the ranges of water quality parameters, based on eight-year dataset, are shown

in Table 1. During the study period, conductivity and Secchi depth averaged $78 \mu\text{S cm}^{-1}$ and 2.2 m, respectively. TN and TP averaged 1.602 mg L^{-1} (range : $0.831\text{--}4.171 \text{ mg L}^{-1}$) and $53 \mu\text{g L}^{-1}$ (range :

$2\text{--}604 \mu\text{g L}^{-1}$), respectively. Concentrations of TN in this system were much greater than the phosphorus content and 85% of the total N was in dissolved inorganic form. Mass ratios of TN : TP

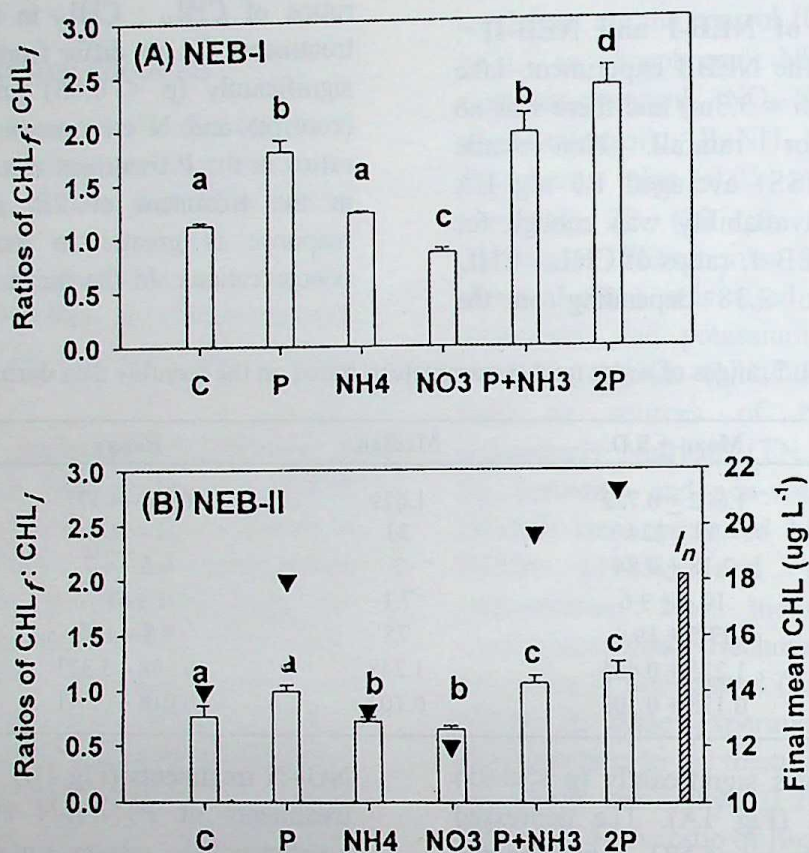


Fig. 1 : Effects of nutrients additions on the algal response, measured as a ratio of final CHL (CHL_f) : initial CHL (CHL_i) in the NEB-I (A) and NEB-II (B). The experimental conditions are the control (C) and treatments of phosphorus (P), ammonia-N (NH_4), nitrate-N (NO_3), phosphorus plus ammonia-N ($\text{P} + \text{NH}_4$) and 2-fold phosphorus (2P). Characters of "a", "b", "c", and "d" indicate a significant difference in the level of $p < 0.05$, based on one-way ANOVA Duncan's multiple range test (SAS, 1991). In the NEB-II, dark triangles and shaded bar (I_n) indicate final mean CHL in each experimental condition and initial concentration of CHL in the ambient water, respectively.

averaged 27, respectively, indicating a potential phosphorus limitation for phytoplankton growth, based on the criteria of Forsberg and Ryding (1980).

Interannual variations : Water quality showed high interannual fluctuations at a given sampling location. Long-term trend of $\text{NO}_3\text{-N}$ showed a distinct increasing phase over the eight year period from 1993 to 2000 and had a similar pattern with TN (Fig 2). The similar patterns between $\text{NO}_3\text{-N}$ and TN were probably attributed to major contribution of nitrate-N to the total nitrogen pool.

Water clarity, measured as secchi depth (SD), also declined consistently from $> 3\text{m}$ in 1993 to $< 2.4 \text{ m}$ in 2000, while conductivity increased $> 40\%$ over the study period. It is evident that eutrophication processes in the system have been rapidly accelerated over the period, based on the parameters of $\text{NO}_3\text{-N}$, TN, transparency, and conductivity. In the mean time, TP, CHL, and TSS, and $\text{NH}_4\text{-N}$ varied yearly and the trends of increasing or decreasing were not clear (Fig 2). Values of CHL had a distinct difference between the high flood year of 1993 and drought year of 1994. Values of CHL in 1993 averaged $5.3 \mu\text{g L}^{-1}$,

Determination of a limiting nutrient regulating algal biomass.

was minimum among eight years, and the variation by site was lowest over the study years. Thus, trophic state, based on the CHL of 1993, was mesotrophic, based on the criteria of Forsberg and Ryding (1980), while the mean of 1994 was 16.3

$\mu\text{g L}^{-1}$, indicating a eutrophic condition. Such trophic difference reflected hydrodynamic characteristics. The reduced CHL in 1993 was probably due to a washing-out of phytoplankton cell from the water column by rapid flushing, while

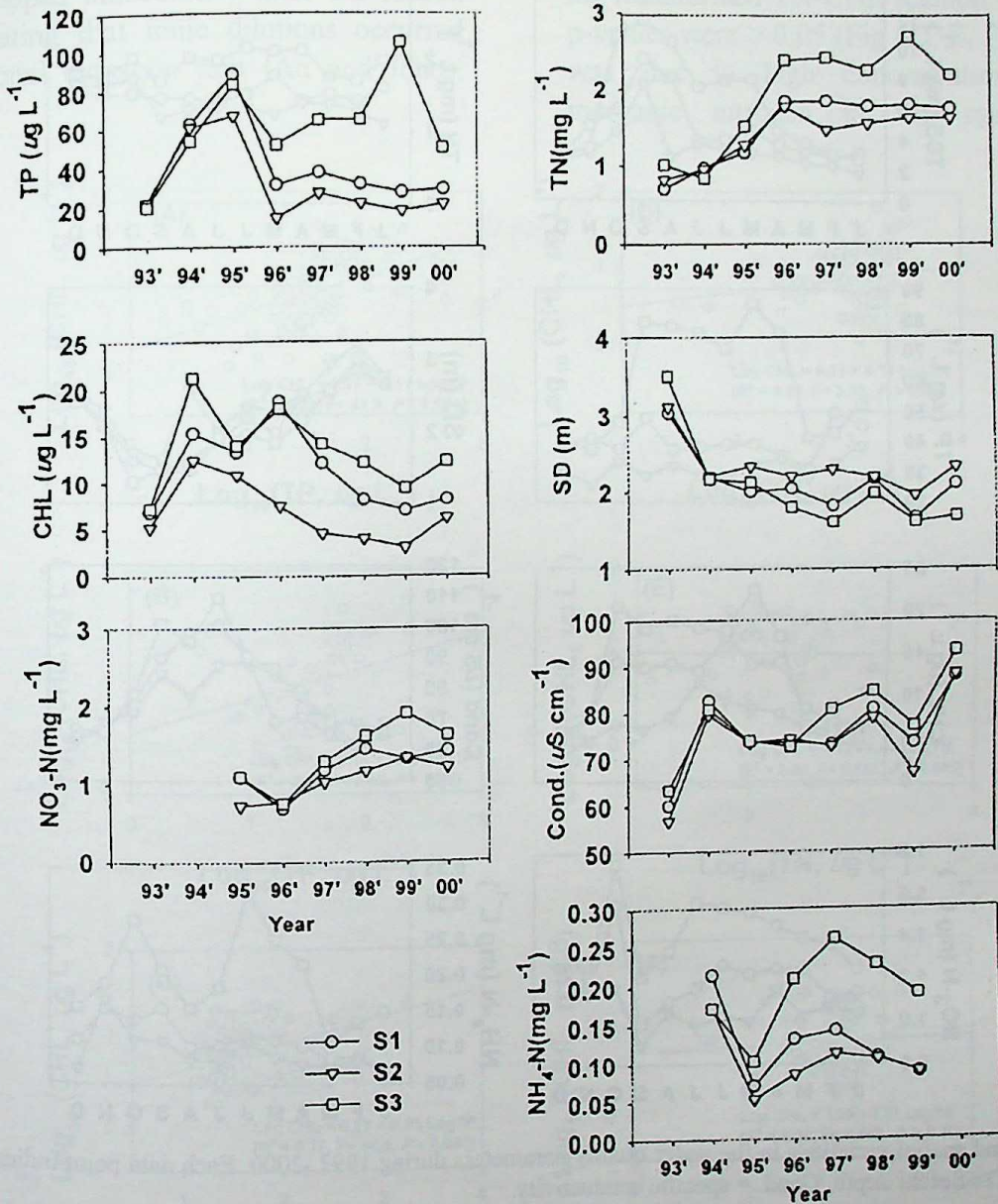


Fig. 2 : Long-term annual variation of water quality parameters, measured at the three locations during 1993 -2000. Each data point indicates an average of 8 years. SD= Secchi depth, Cond. = specific conductivity.

the high CHL in 1994 was due to greater yield of CHL at a unit of given nutrient during long water residence time. These outcomes suggest that CHL and TP seem to vary depending on interannual physical disturbance in response to the hydrological regime.

Seasonal and spatial variations : Monthly values, based on mean values of eight year, showed various seasonal patterns depending on sampling sites and variables used. Long-term monitoring of all seven variables indicated that seasonal water quality condition was greatest in the S2 and worst

in the S3 (Fig 3). Poor water quality in the S3 reflected the discharge water from Soyang dam in the southern river and greatest conditions in the S2 was an influence of the discharge from Chuncheon

dam in the northern river. For this reason, S1 near the dam had mid-level condition in the water quality as a result of the mixing of S2 and S3 waters. Mean TP in the S3 showed marked

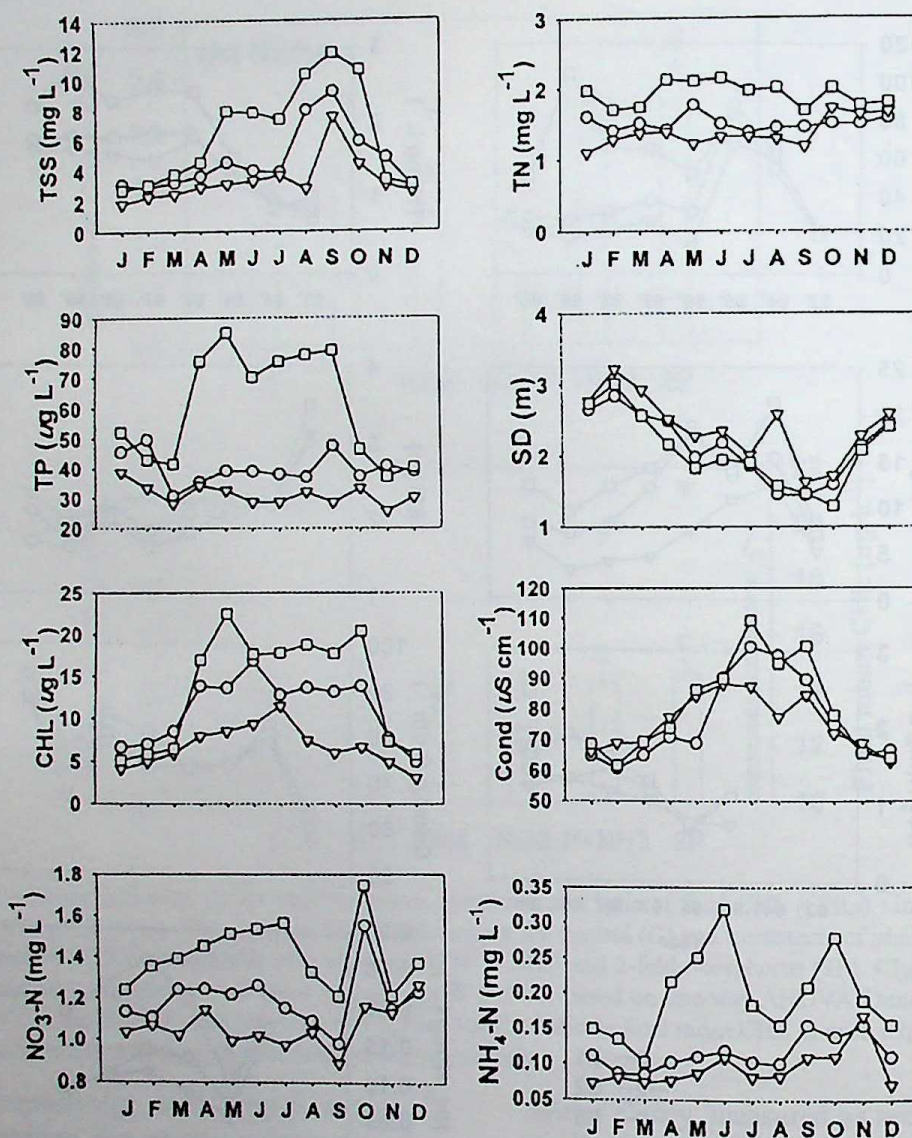


Fig. 3 : Seasonal and spatial variations in the water quality parameters during 1993 -2000. Each data point indicates an average of 8 years. SD= Secchi depth, Cond. = specific conductivity.

difference of $> 30 \mu\text{g L}^{-1}$, especially during all seasons and were significantly ($p < 0.05$) lower than those of S1 and S3. Similar patterns were found in TN, $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ values. Previous studies pointed out that nitrogen, phosphorus, and conductivity were greater in the southern part than in the northern part. Therefore, high TP in the S3 was probably due to arrivals of nutrient-rich water from the meta-to hypolimnetic discharge of the

upper dam. Such impacts of up-reservoirs are demonstrated in the serial impoundments of North America and Europe (Petts, 1984; Ward Stanford, 1983). In the mean time, total suspended solids (TSS) were greater in the period of August-October than other periods. This was probably due to a combined effect of increased algal biomass and inorganic solids. Thus, transparency had maximum in winter and minimum during August-

Determination of a limiting nutrient regulating algal biomass.

October, indicating that mineral turbidity (i. e., inorganic solids) and organic matters (phytoplankton cells) are low in the winter and high during the period of summer to fall. Monthly mean conductivity peaked in the July-August period and dropped immediately after the season (Fig 3), indicating that ionic dilutions occurred after the seasonal monsoon rain (An and Jones, 2000).

Empirical relations of CHL-TP : Regression analyses of log10-transformed CHL against nutrients showed that phytoplankton growth was not influenced by nitrogen contents and weak relations to flux of phosphorus. Values of R^2 in the log-transformed TN-CHL relation were < 0.04 and p-values were > 0.05 (Fig 4D, E, F). This situation was due to high concentrations of ambient inorganic nitrogen in the system and this

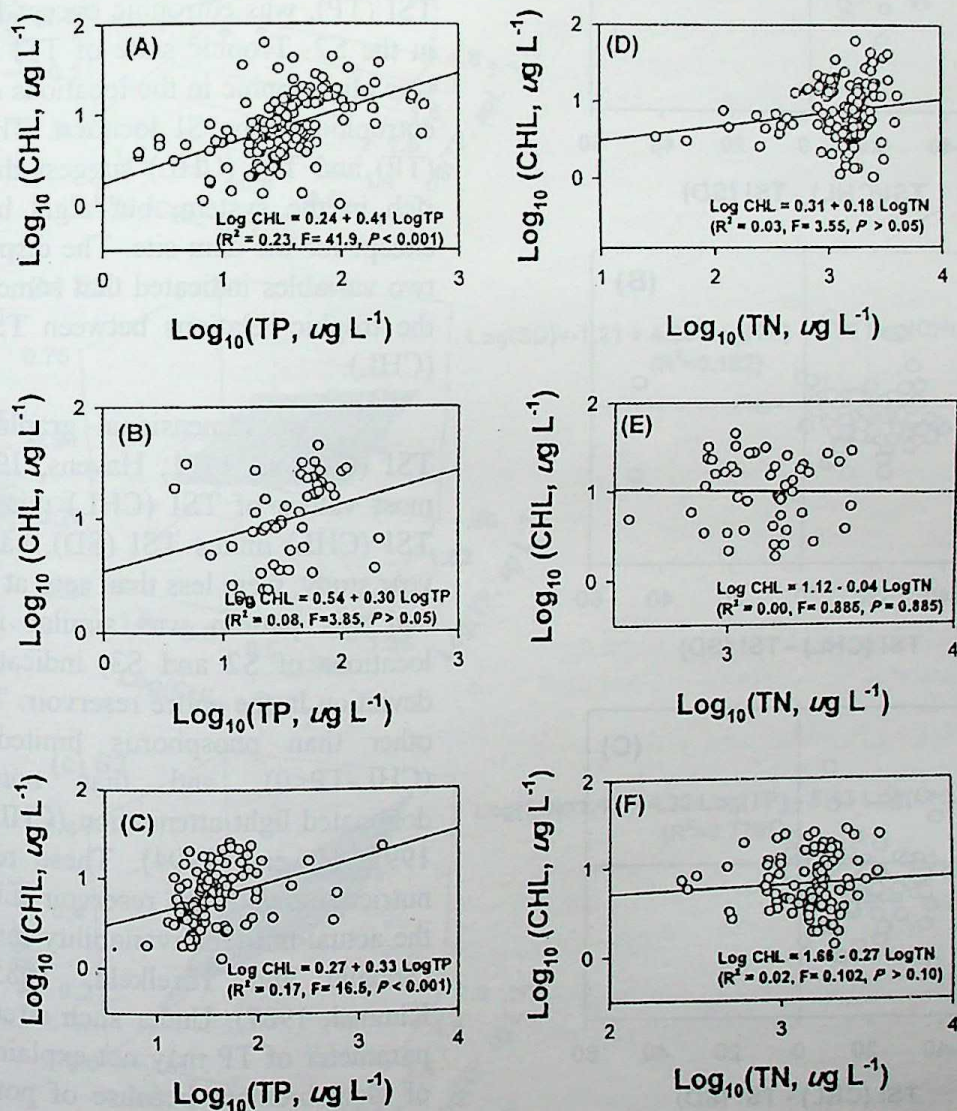


Fig. 4 : Empirical relations of seasonal log₁₀-transformed CHL-TP (A-C) and CHL-TN (D-F). Figures of (A) & (D), (B) & (E), and (C) & (F) are based on the premonsoon, monsoon, and postmonsoon data, respectively.

supposition was explained by *in situ* manipulation experiments of NEB-1 and NEB-II. In the mean time, there were weak relations between CHL and TP (R^2 values < 0.25) during premonsoon and postmonsoon seasons, while the relation was not

significant ($R^2 = 0.08, p > 0.05$) during monsoon period (Fig 4A, B, C). The ranges of R^2 were much lower than in other Korean systems of Soyang (Lee *et al.*, 2002) and Taechung reservoirs (An and Park, 2002), but no relations of CHL-TP

during summer monsoon were same as the previous studies. This difference was probably attributed to short hydraulic residence time (HRT)

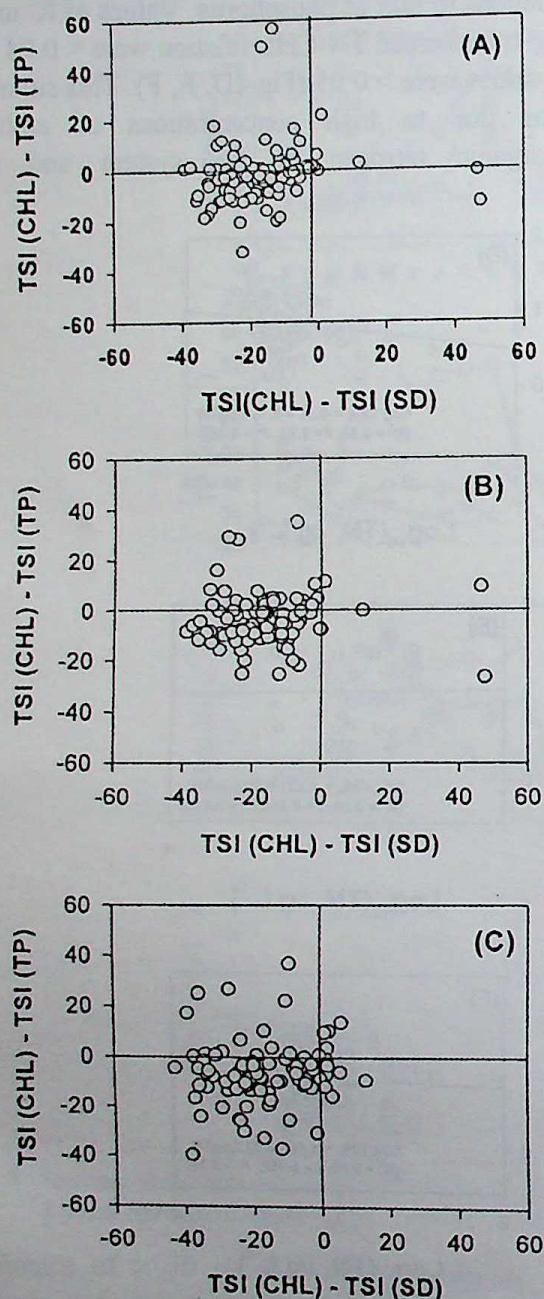


Fig. 5 : Trophic relations between TSI (CHL) - TSI (TP) and TSI (CHL) - TSI (SD) in the premonsoon (A), monsoon (B), and postmonsoon season (C).

of 5 -17 day in the Uiam reservoir, compared to the other systems (i. e., Soyang and Taechung reservoirs; mean HRT > 100 day). In this system, the HRT seems to be the most important factor

modifying the relations between CHL and TP variables, as shown especially during monsoon seasons in other reservoirs (An and Jones, 2002; An and Park, 2002).

Relations among trophic parameters : Trophic conditions, measured as the Trophic State Index (Carlson, 1977, 1991), varied depending on the year, trophic variable, and the location of the reservoir. Trophic state, based on the criteria of TSI (TP), was eutrophic except for a short period in the S2. Trophic state of TSI (CHL), however, was oligotrophic in the locations of S2 and S3 and eutrophic in the SI location. The values of TSI (TP) and TSI (CHL) suggest that phosphorus is rich in the system, but algal biomass was low except for the dam site. The disparity between the two variables indicated that some factors modified the trophic relations between TSI (TP) and TSI (CHL).

Two dimensional graphical approach of TSI (Carlson, 1991; Havens, 1994) showed that most values of TSI (CHL) minus TSI (TP) and TSI (CHL) minus TSI (SD), based on the eight-year study, were less than zero at the location of SI and this pattern was similar in the remaining locations of S2 and S3, indicating trophic state deviation in the entire reservoir. Therefore, factors other than phosphorus limited algal biomass ($\text{CHL-TP} < 0$), and that non-algal particles dominated light attenuation ($\text{CHL-SD} < 0$; Carlson, 1991; Havens, 1994). These results imply that nutrient inputs to the reservoir substantially exceed the actual nutrient availability for algal production (Soballe and Threlkeld, 1985; Soballe and Kimmel, 1987). Under such circumstances, single parameter of TP may not explain the all variation of algal biomass because of potential large non-algal light attenuation and rapid flushing (Lind *et al.* 1993; Havens 1994), so water renewal rate seems to be another key element regulating algal growth. In the mean time, when transparency, measured as Secchi depth, was expressed as a function of two independent variables, algal biomass and the limiting nutrient influenced water clarity (Fig 6). Multiple regression analyses of

Determination of a limiting nutrient regulating algal biomass.

\log_{10} -transformed SD showed that TP and CHL accounted 77.8% for the variation of transparency in the location of S3 and 41% for the transparency in the location of S1 (Fig 6). This analysis

indicates that the limiting nutrient of phosphorus contributed partially to phytoplankton growth and this, in turn, influenced water clarity. In contrast, such effect was weak in the S2. These findings

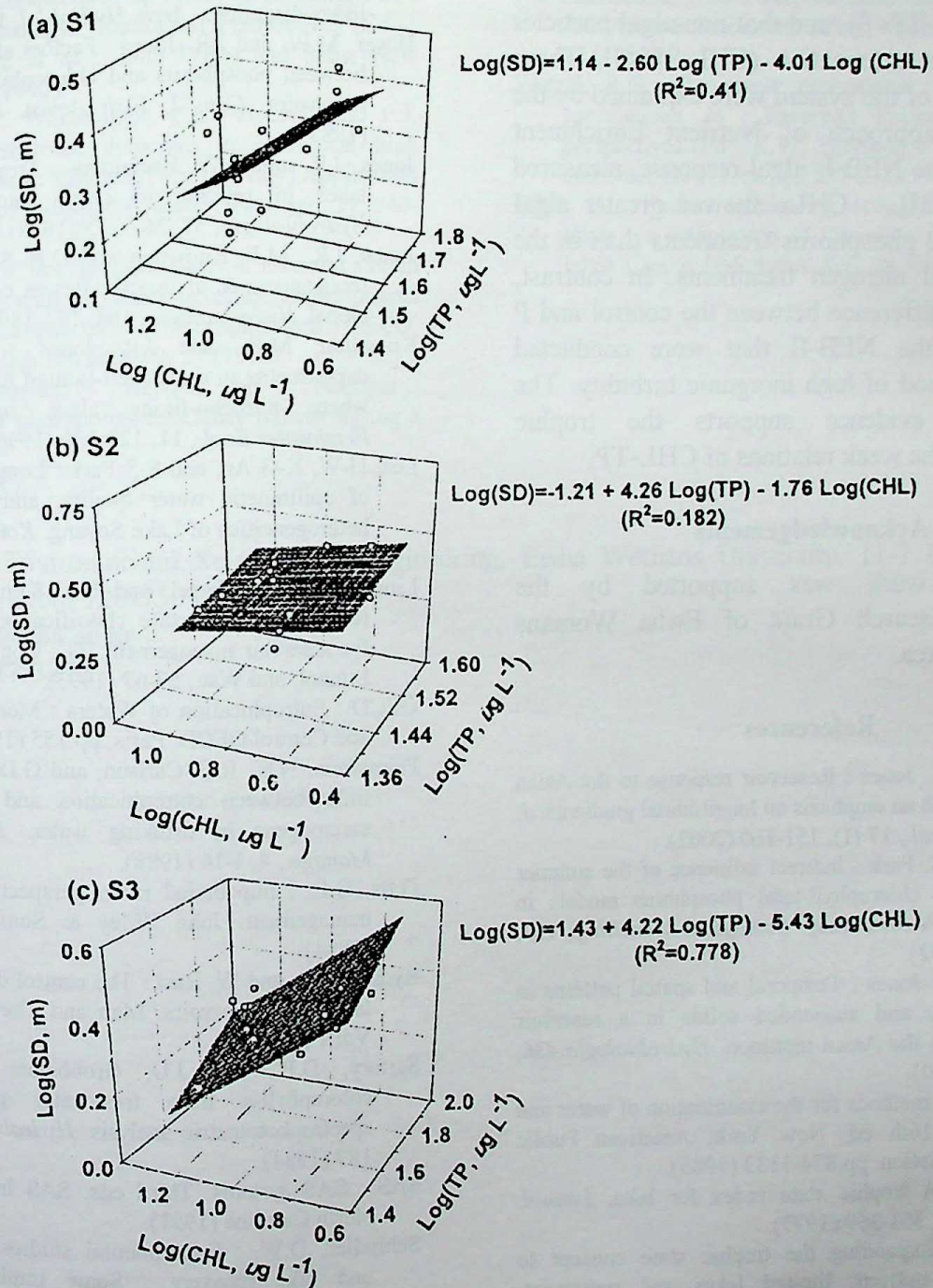


Fig. 6 : Multiple regression analysis of Secchi depth (SD) as a function of CHL and TP in the S1(A), S2(B), and S3 locations (C).

suggest that trophic deviation is evident in the system, but still water clarity reflects concentrations of algal biomass and the limiting nutrient.

Overall, ambient nutrient contents and nutrient ratios indicated that potential phosphorus limitation was evident. Empirical relations of CHL-TP, however, were low based on the log-

transformed regression analyses and these relations were highly influenced by seasonal monsoon and interannual rainfall. Two-dimensional graphical approach of trophic state index suggested that factors other than phosphorus limited algal biomass ($CHL-TP < 0$), and that non-algal particles dominated light attenuation ($CHL-SD < 0$). These characteristics of the system were explained by the experimental approach of Nutrient Enrichment Bioassay. In the NEB-I, algal response, measured as ratios of $CHL_i : CHL_f$, showed greater algal response in the phosphorus treatments than in the control and all nitrogen treatments. In contrast, there was no difference between the control and P treatment in the NEB-II that were conducted during the period of high inorganic turbidity. The experimental evidence supports the trophic deviation and the weak relations of $CHL-TP$.

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Identification of ecologically significant habitats for urban nature conservation : A case study in Turkey.

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Abstract : *Given the rapid urbanization of Turkey, sustained productivity of natural resources should be an integral part of any urban development policy. The biogeochemical cycles and ecosystem services link urban and rural ecosystems. It is, therefore, essential that ecologically significant habitats along urban-rural continuum be protected to secure public and environmental health. This necessitates their identification and the establishment of administrative and legal foundations for urban nature conservation in the management of urban habitats. should be established. Ecological analysis of urban habitats of Karşıyaka led to the identification of 19 ecologically significant habitats. Nature conservation priority was rated, using rarity, species richness, stratification, site age, and area of the habitats. Ecologically significant habitats made up about 54 ha of the total urban green space of 289 ha in Karşıyaka. The total number of plant species was estimated to be 273.*

Key words : *Urban ecology, Nature conservation, Sustainable development, Environmental policy.*

Introduction

A rapid growth of human population and consumption, and ecologically incompatible land- and energy-use decisions based solely on economic considerations increasingly degrade the structure and function of rural and urban-industrial ecosystems (Evrendilek and Doygun, 2000). The resultant environmental problems include pollution of air, water and soil, losses of biodiversity and prime farmlands, and fragmentation and destruction of ecologically significant habitats (Botkin and Beveridge, 1997; Evrendilek and Ertekin, 2002). Local environmental degradation due to urbanization and industrialization, in turn, contributes to such regional and global environmental changes as climate change, acid deposition, depletion of stratospheric ozone layer, and biological invasions (Vitousek et al., 1997; Wali et al., 1999). Therefore, there is an urgent need for a holistic approach of urban nature conservation, particularly in rapidly urbanizing countries (Sukopp 1990).

Urban human population of the world increased to 50% in the year 2000 and is expected to increase to more than 66% by 2025

(Brockhoff, 1996). Urban population in Turkey increased from 3 million in 1950 (14.4% of the total population) to 47.5 million (70.6%) in 2000 and is projected to reach 79, 681 (87.2%) by 2030 (FAO, 2001). Urban corridors, woodlands, greenways, parkways, parks, gardens, orchards, wetlands, and riparian zones are the major representatives of ecologically significant habitats in urban areas. Ecologically significant habitats provide such ecosystem goods and services as primary and secondary production, regulation of hydrological and micro climatic conditions, sequestration of greenhouse gases, shelter and protection for organisms, assimilation of wastes, pollutants and noise, integrity of habitats, conservation of biological diversity, enhancement of environmental awareness, recreational values, and biological indicators for environmental health (McDonnell and Pickett, 1990).

The purpose of this study was to assess ecologically significant habitats of the urban settlement of Karşıyaka (İzmir) and processes by which long-term sustainability of ecosystem goods and services provided by the habitats are secured and threatened.

Materials and Methods

Study area : The urban area of Karşıyaka (27°05'-27°11'E and 38°27'-38°30'N) comprises about 5,500 ha. Karşıyaka was established in the late 19th century on the north alluvial plains of İzmir Bay and is surrounded by İzmir Bay in the south, Yamanlar mountain (1076 m) in the north, Çiğli alluvial plains in the west, and Bornova alluvial plains in the east. Parent materials of Yamanlar mountain consist of igneous rocks from Neocene time, and the plains consist of Quaternary alluvial materials. The study area has two intermittent streams that spring from 150-200 m of Yamanlar mountain : Kocadere and İlica.

The prevalent climate regime of Karşıyaka is the Mediterranean climate characterized by mild winters (during which at least 65% of the average annual precipitation of 677 mm falls) and hot dry summers (during which at least 75% of the average annual potential evapotranspiration of 900 mm occurs). The average annual temperature is 17.5°C with the average minimum and maximum temperatures of 5.6°C in January and 32.9°C in August, respectively.

Sclerophyllous vegetation of the mediterranean phytogeographical domain is the dominant vegetation of the study area. Floristic analysis of Yamanlar mountain revealed that there were 725 vascular plant taxa out of which the number of endemic taxa were 25 (3.4%) (Gemici and Seçmen, 1983). Vegetation of Yamanlar

mountain showed variations in its composition and distribution along an altitudinal bioclimatic gradient. The bioclimatic zones and their plant sociological classifications were as follows (Gemici and Seçmen, 1983) :

Thermo-Mediterranean zone (0-200 m) with associations of *Sarcopotarium spinosum*, *Quercus coccifera* and *Pinus brutia*,

Eu-Mediterranean zone (200-800 m) with the same associations and sub-association of *Asphodelus microcarpus*, and

Subra-Mediterranean zone (800-1000 m) with associations of *Pinus nigra* sub sp. *pallasiana* and *Onopordetum illyrici* and sub-associations of *Chondrilla juncea* var. *juncea* and *Verbascum lasianthum*.

In the course of field observations, the following variables were taken into account in the identification of ecologically significant habitats of Karşıyaka : location; topography; spontaneous and planted vegetation; actual land use; a real extent; site age; species richness; abundance, cover, sociability, stratification and tallest tree of vegetation; and rarity (Wittig and Schreiber, 1983; Sukopp and Weiler, 1988). Urban habitats were sampled through the representative quadrates to obtain values of species cover-abundance and sociability according to the Braun-Blanquet's scale (1932) (Table 1). Rarity, stratification, a real extent, site age, and species richness were

Table - 1 : Braun-Blanquet's system of assessing vegetation (Braun-Blanquet, 1932).

Scale	Cover-abundance	Sociability
+	(very) sparsely present, covering <1%	
1	plentiful, covering 1-5%	
2	very numerous, covering 6-25%	growing singly
3	any number of individuals covering 26-50%	grouped or tufted
4	any number of individuals covering 51-75%	in small patches or cushions
5	any number of individuals covering 76-100%	in small colonies or in extensive patches
		in pure populations

measured based on a standardized scale ranging from 0 (least possible value) to 5 (highest possible value) (Table 2). The variables in the rating of ecological significance and conservation priority of urban habitats were of equal weight.

Simple linear regression models were performed to relate the response variable of conservation value of ecologically significant urban habitats to the explanatory variables of rarity, stratification, area, site age, and species

Urban nature conservation.

richness. Correlation matrix (the Pearson's correlation coefficient, r) between each pair of the variables was calculated to investigate the relatedness of the variables. All statistical analyses were conducted with Minitab 12.1.

Results and Discussion

Ecological assessment of habitats of the urban settlement of Karşıyaka and its surrounding revealed a total of 19 ecologically significant habitats composed of 15 human-managed and 4

Table - 2 : Scale of rarity, stratification, area, site age, and species richness for quantification of urban nature conservation priority.

Scale	Rarity (distance from the nearest similar habitat)	Stratification	Area (ha)	Site age (year)	Species richness (number)
0	≤ 500 m	trampled grass layer	≤ 0.5	≤ 5	≤ 15
1	500-1000 m	grass layer with scattered trees	0.6-1	5-10	15-25
2	1-3 km	tree layer with little grass	1-2	10-20	25-35
3	3-5 km	two separate layers	2-5	20-50	35-50
4	5-7 km	three separate layers	5-10	50-70	50-70
5	> 7 km	> three separate layers	> 10	> 70	> 70

semi-natural ones (Table 3). The plant sociological description of the habitats was shown in Table 4. Ecologically significant urban habitats made up about 54 ha (19%) of the total urban greenspace of about 289 ha. Cemeteries of Soğukkuyu and Ömekköy, parks of Yamanlar and Orphanage, and hedgerows of horticultural land received the highest ratings for nature conservation priority (Table 5). Species richness of Karşıyaka was estimated to be 273 out of which woody and non-woody species were 121 and 152, respectively. Similarly, the total number of plant species for the mediterranean areas of California was estimated to be 307 (Shmida, 1981). Although terrestrial ecosystems with the mediterranean climate occupy 1.7% of the earth's surface area, mediterranean ecosystems support high biological diversity and endemism (Lenz and Dourley, 1981). Prevailing semi-arid conditions make the mediterranean ecosystems extremely vulnerable to and unable to recover from drastic human-induced disturbances unless preventive and mitigative measures are taken against the threats to their present and future well-being.

Simple linear regression models indicated that the explanatory variables of species richness, rarity, and area were of great importance in accounting for variation in conservation priority of the urban habitats ($P < 0.001$) (Table 6).

According to the correlation matrix of the variables, there were strong positive relationships between species richness and rarity, and species richness and stratification ($P < 0.005$) (Table 7). The positive relationship between species richness and stratification revealed that an increase in the stratification of vegetation as in the successional development assists in the sustainable management of the urban habitats. Planting of indigenous species decreases uses of biocides, irrigation and fertilizers, and the establishment chance of invasive species, thus lowering maintenance costs of the urban habitats. Indigenous species also supply food sources for wildlife, and hence, is more amenable to sustaining wildlife than exotic species (Cicero, 1989).

Misuses, overuses, and incompatible uses of land and water resources, with the resultant environmental impacts on urban and (semi) natural habitats of Karşıyaka were outlined in Table 8. Urban sprawl of Karşıyaka has resulted in an irreversible loss of productive horticultural lands, orchards and vineyards. Remnants of these agricultural lands along highways have been adversely affected by the accumulation of heavy metals in soils, and air pollution caused by heavy traffic. Thus, the resultant decrease in agricultural productivity and produce quality has impaired public health and economic well being.

Clear-cut and burning of forest areas to provide urban residential areas have resulted in the losses and degradation of steady-state ecosystems of Yamanlar mountain (e.g., *Pinus brutia* and *P.*

nigra sub. *Pauasiana*), which caused the losses of such important ecological functions as the prevention of soil erosion, flooding, and purification of water, soil and air, and the

Table - 3 : Ecological description of human-managed and natural habitats of Karşıyaka.

Habitat type	Human-managed habitats															(Semi) natural habitats			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Area (ha)	20	1.5	0.8	2	3	1	1	13	1.5	0.6	0.5	0.5	0.5	3	5		100-200		
Site age (yr)	50	6	30	50	40	40	40	70	50	40	40	40	40	40	40		70-100		
Altitude (m)	50	0	5	5	0	6	0	20	20	20	10	5	5	5	10	250	400	120	920
Aspect	S	L	L	L	L	L	L	L	L	L	L	L	L	L	L	SW	NE	SE	NW
Slope (%)	40	2	2	2	2	1	1	2	2	2	5	2	2	2	5	40	25	45	5
Parent material	An	A	A	A	A	A	A	A	A	A	A	A	A	A	A	An	An	An	An
Soil group	BC	A	A	A	A	A	A	A	A	A	A	A	A	A	A	BC	BC	BC	BC
Stratification	3	3	3	3	2	1	1	3	3	2	2	1	2	2	1	2	2	4	4
Tallest tree (m)	12	10	15	20	20	25	10	20	8	8	12	12	20	12	12	0.5	2	20	20
Total cover (%)	70	100	80	100	100	25	25	100	100	20	50	100	85	90	20	90	65	70	55
Total woody cover (%)	40	40	80	55	50	25	25	85	75	20	25	10	65	70	5	90	65	70	55
Planted vegetation (%)	35	80	>95	60	>95	>95	>95	60	20	35	20	25	70	10	25			<5	
Spontaneous vegetation (%)	65	20	<5	40	<5	<5	<5	40	80	65	80	75	30	90	75			>95	
Species richness	59	55	31	73	47	21	17	55	19	27	63	20	40	32	39	68	50	46	44

Names of habitats 1 to 7 are Parks of Yamanlar, Muammer Aksoy, Zübeyde Hanım, Orphanage, Regional Directorate of Forestry, and Karşıyaka Gazi and Professional High School, habitats 8-9 Cemeteries of Sogukkuyu and Ornekkoy, habitat 10 Hedgerows, habitat 11 Ilica creek banks, habitat 12 Vacant spaces, habitat 13 Karşıyaka railway, habitat 14 Naldoken orchards, habitat 15 Hedgerows of horticultural gardens, habitat 16 Phrygiana, habitat 17 Maquis, habitat 18 *Pinus brutia* association, and habitat 19 *Pinus nigra* sub. *pallasiana* association. An, Andesite; A, alluvial; and BC, brown calcarousless soils.

provision of recreational and aesthetical values, and biodiversity. The Reminders of *Pinus brutia* and *P. nigra* forests together with phrygiana and maquis formations should therefore be protected for the sustained biogeochemical maintenance of ecosystem goods and services that satisfy local human needs for the present and future generations. Kocadere and Ilica streams originating from Yamanlar mountain have been so degraded by uncontrolled disposal of residential and industrial wastes that they have become aesthetically unpleasant and a source of bad odor.

Wetlands along İzmir Bay were drained and filled up to be converted into residential, agricultural and waste disposal areas. This has caused such ecological services provided by the wetlands as migration routes for birds, regulation of hydrological cycle, biodiversity, and assimilation of water pollutants to be lost irreversibly. Chemical and food industries located along İzmir Bay have caused a substantial decrease in water quantity and quality due to their excessive water use and disposal of untreated wastes. Ecologically improper waste disposal and vast landfills (10-20

Urban nature conservation.

m wide) have destroyed aquatic life, altered natural pattern of drainage and impoverished recreational value in the Bay.

Population of Karşıyaka increased rapidly at an annual rate of 19.2% from 53,372 in 1955 to 432,457 in 1997. Currently, Karşıyaka has a total

Table - 4 : Plant sociological description of human-managed and natural habitats of Karşıyaka (frequency level of greater than 15%).

Habitat type	Human-managed habitats															(Semi)natural habitats				Frequency
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Woody species																				
<i>Nerium oleander</i>	+1	+1	+1	+1	+1	+1	+1	+1			+1	+1	+1		+1					63%
<i>Morus alba</i>	+1		31	11		11	+1	+1	+1	+1	+1		11	+1	+1					63%
<i>Pistacia lentiscus</i>	+1			21		11	+1	11	41	+1	+1		+1		+1					53%
<i>Cupressus sempervirens</i>	+1		+1	11	11	21	+1	31	21		+1		+1							53%
<i>Phoenix dactylifera</i>	+1	11	+1	+1	+1	+1	11						+1	+1						47%
<i>Pinus pinea</i>		+1	+1	11	+1		+1	+1		+1			11	+1						47%
<i>Ailanthus altissima</i>	+1	+1		+1		11	+1	+1			11		+1							42%
<i>Casuarine aquisetifolia</i>	+1	+1	+1		+1	11		+1			+1	11								42%
<i>Eucalyptus camaldulensis</i>		11	+1		+1	+1				+1	+1		+1		+1					42%
<i>Washingtonia robusta</i>		+1	+1	11	+1		11	+1							+1					37%
<i>Eunonymus japonica</i>	+1	+1	+1	+1	+1	+1		+1												37%
<i>Ligustrum vulgare</i>			11	+1	+1	+1	+1	+1					+1							37%
<i>Ficus carica</i>	+1			+1		+1		+1		+1	+1				+1					37%
<i>Pinus brutia</i>	+1			+1	+1	31		41	+1									44		37%
<i>Acacia cyanophylla</i>		11	11			+1		+1			+1		+1							32%
<i>Schinus molle</i>		+1	+1		+1	+1	+1						+1							32%
<i>Jasminium officinalis</i>	+1	+1	+1	+1			+1	+1												32%
<i>Pyracantha coccinea</i>	+1	+1	+1	+1	+1	+1														32%
<i>Cupressus arizonica</i>	+1	+1	+1	+1	+1	+1														26%
<i>Laurus nobilis</i>		+1	+1	+1				+1					+1							26%
<i>Citrus sp.</i>										+1			+1	51	+1					26%
<i>Magnolia grandiflora</i>	+1		+1	+1	+1								+1							26%
<i>Salix babylonica</i>		+1	+1	+1		+1						+1								26%
<i>Rosa sp.</i>		+1	+1	+1	+1		+1													26%
<i>Populus euamericana</i>				21	+1						+1	11			+1					26%

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Urban nature conservation.

<i>Erodium malacoides</i>	±1							±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	53%	
<i>Hordeum bulbosa</i>	±1		33					±1	32	±1	22		12	22		±1	±1	53%	
<i>Senecio vulgaris</i>	±1	11	±1					±1	±1		11		±1	±1	±1	±1		53%	
<i>Poa bulbosa</i>	42		22					±1	22	±1	22			43	±1	±1	11	53%	
<i>Trifolium angustifolium</i>	32	43	22							±1	±1	33	22	±1			11	47%	
<i>Taraxacum serotinum</i>	±1	31	±1					±1			±1		±1	±1				42%	
<i>Geranium molle</i>	±1		±1					±1	±1		±1					11		37%	
<i>Capsella bursa-pastoris</i>	±1									±1	±1	±1	±1	11	±1			37%	
<i>Matricaria chamomela</i>	±1	±1						±1		±1	12	±1				22		37%	
<i>Sinapis alba</i>	±1								11	±1	±1	±1		±1	±1			37%	
<i>Malva sylvestris</i>		±1	±1					±1	±1	±1	±1	±1						32%	
<i>Trigonella sp.</i>	22		12					±1			11			±1	±1			32%	
<i>Crepis zacintha</i>	±1	11	±1								11		±1		11			32%	
<i>Bromus diandrus</i>	±1							±1			42	42	12					26%	
<i>Vicia villosa</i>	±1		42					±1						22	±1			26%	
<i>Veronica cymbalaria</i>	±1											±1		11	±1		±1	26%	
<i>Lamium amplexicaule</i>										±1		±1	±1	11		±1		26%	
<i>Sedum confertiflorum</i>	±1							±1					±1	±1		±1		26%	
<i>Sisymbrium altissimum</i>	±1										22	41		42	11			26%	
<i>Stellaria media</i>			±1					±1			12	11					±1	26%	
<i>Asphodelus microcarpus</i>	±1								41						±1	±2	11	26%	
<i>Anthemis arvensis</i>								±1		±1				31	±1	11		26%	
<i>Verbascum sinuatum</i>	±1	±1								±1					±1			21%	
<i>Calendula arvensis</i>			±1					±1		±1						±1		21%	
<i>Galium aparine</i>			±1					±1		±1	13							21%	
<i>Ranunculus marginatus</i>	11		11								±1			21				21%	
<i>Lolium preenne</i>															±1	±1		21%	
<i>Urtica urens</i>	±1		±1												±1	±1		21%	
<i>Polygonum maritimum</i>			22								±1				±1	±1		21%	
<i>Alsium fluescens</i>														±1		11	11	±1	21%
<i>Papever rhoeas</i>	±1									±1					±1	±1		21%	
<i>Daucus carota</i>	±1										32				12			16%	
<i>Euphorbia helioscopia</i>			±1											31	±1			16%	
<i>Plantago lanceolata</i>	11	31	11															16%	

.....continued to page

<i>Ranunculus</i>	11	11	11	16%
<i>muricatus</i>				
<i>Avena barbata</i>		+1	11 +1	16%
<i>Ballota</i>		+1	12 12	16%
<i>acetobulosa</i>				
<i>Sherardia</i>			11 11 +1	16%
<i>arvensis</i>				
<i>Hymnocarpus</i>			+1 11 +1	16%
<i>circinnatus</i>				
<i>Lagoecia</i>			11 +1 +1	16%
<i>cuminoides</i>				
<i>Trifolium</i>			+1 11 +1	16%
<i>stellatum</i>				
<i>Torilis arvensis</i>			+1 11 +1	16%
<i>Rumex</i>	21		11 +1	16%
<i>bucephalophorus</i>				
Total woody species				121
Total non-woody species				152
Total				273

Woody species with a frequency level of less than 15%: *Cotoneaster horizontalis*, *Acer negundo*, *Albizia julibrissima*, *Brachichiton populneum*, *Berberis thunbergii*, *Cestrum purpurea*, *Inula viscosa*, *Hedera helix*, *Juniperus sabina*, *Hibiscus syriacus*, *Parthenocissus quinquefolia*, *Picea orientalis*, *Lagerstromia indica*, *Rosmarinus officinalis*, and *Sambucus nigra*; and non-woody species with a frequency level of less than 15%: *Allium scorodoprassum*, *Mentha pulegium*, *Alopecurus myosuroides*, *Salvia fruticosa*, *Chenopodium album*, *Eryngium smyrnaeum*, *Muscari comosum*, *Oryzopsis coerulescens*, *Carduus pycnocephalus*, *Bromus tectorum*, *Aira capillaries*, *Tordylium apulum*, *Galium caudatum*, *Dactylis glomerata*, *Euphorbia peplus*, *Rumex tuberosus*, *Cynosourus echinatus*, *Origanum onites*, *Stachys cretica*, *Geranium lucidum*, *Briza maxima*, *Trifolium uniflorum*, *Crucianella angustifolia*, *Cuscuta planiflora*, *Sonchus oleraceus*, *Phalaris* sp., *Oxalis pes-capreae*, *Viola odorata*, *Aphanes arvensis*, *Anagallis arvensis*, *Crepis foetida*, *Plantago logopus*, *Theligonum cynocrambre*, *Parentucellia latifolia*, *Medicago orbicularis*, *Veronica arvensis*, *Filago eriocephala*, *Trifolium scutatum*, *Tuberaria guttata*, *Lepidium spinosum*, *Trifolium subterraneum*, *Trifolium pilulare*, *Erodium cicutarium*, *Plantago afra*, *Scandix pecten*, *Plantago coronopus*, *Bunias ericago*, *Plantago cretica*, *Anthoxanthum odoratum*, *Anthemis wiedmanniana*, *Trifolium scabrium*, *Bromus scoparius*, *Bromus sterilis*, *Scleropa rigida*, *Trifolium arvense*, *Alyssum smyrnaeum*, *Umbilicus rupestris*, *Ranunculus arvensis*, *Parietaria lusitanica*, *Tamus communis*, *Rumex pulcher*, *Srenaria serpyllifolia*, *Trifolium campestre*, *Cerastium illyricum*, *Aegilops ovata*, *Trifolium resupinatum*, *Trifolium globosum*, *Helianthemum aegyptiacum*, *Lusimachia linum*, *Aristolochia hirta*, *Valerianella obtusiloba*, *Biserrula pelecinus*, *Medicago disciformis*, *Melica ciliata*, *Orlaya daucoides*, *Trachynia distachya*, *Lolium tumulentum*, *Petrorrhagia velutina*, *Stipa bromoides*, *Ornithopus compressus*, *Micromeria nervosa*, *Valantia hispida*, *Nephelochloa orientalis*, *Theligonum eynocambe*, *Urospermum picioides*, *Tolpis barbata*, *Simphtum anatolicum*, *Anthemis aciphylla*, *Piconomon acarna*, *Rumex acetosella*, *Bromus erectus*, *Vicia cuspidate*, *Vicia articulata*, *Veronica grisebachii*, *Moenchia mantica*, *Myosotis ramosissima*, *Erophila verna*, *Sanguisorba minor*, *Potentilla recta*, *Lathyrus saxatilis*, *Galium caudatum*, *Parentucellia latifolia*, *Tanacetum smyrnaeum*, *Bromis mollis*, *Lathyrus setifolius*, *Ranunculus paludosus*, *Campaluna lyrata*, and *Galium murale*.

open greenspace of 289 ha (equivalent to 5.2% of the total city area) 30.2 ha of which made up public parks (Table 9). Per capita greenspace and public park area were 2.86 m² and 0.85 m², respectively, well below per capita urban greenspace of 7 m² required by City Planning Law no. 3194 of 1972 (Danielson and Keleş 1985). Although Environmental Law no. 2872 of 1983, and National Parks Law no. 2873 of 1983 enunciate the necessity of preventive and mitigative measures towards rational uses of natural resources, there are no clear legal measures and

public policies that take into account the nature conservation of urban habitats.

Given the rate- and stock-limited natural resources of the world, developing sustainability requires that human activities use natural resources and generate wastes without progressively exceeding the productive, assimilative and regenerative capacities of and impairing the biogeochemical integrity of habitats wherever located (Rees, 1997; Pauleit and Duhme, 2000). Integration of nature conservation strategies into regional and urban land use planning is an integral

Urban nature conservation.

component of any sustainable urban development policy (Sukopp et al., 1995). It is essential that habitat links along urban-rural continuum be

established and augmented in bioregional context by the identification and protection of ecologically significant habitats. In this way, ecosystem goods

Table - 5 : Urban nature conservation priority for ecologically significant urban habitats based on weighting factors.

Habitat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Criteria	2	0	0	3	1	0	0	3	3	1	1	0	1	2	3
rarity	4	4	4	5	3	2	2	4	4	3	3	1	1	2	1
stratification	5	2	1	2	3	1	1	5	2	1	1	1	1	3	3
area	5	1	3	4	3	3	3	5	4	3	3	3	3	3	3
site age															
species richness	4	4	2	5	3	1	1	4	4	4	2	1	1	2	4
conservation value															
(out of 25)	20	11	10	19	13	7	7	21	17	12	10	6	7	12	14

Names of habitats 1 to 7 are Parks of Yamanlar, Muammer Aksoy, Zübeyde Hanım, Orphanage, Regional Directorate of Forestry, and Karşıyaka Gazi and Professional High School; habitats 8 to 9 Cemeteries of Soğukkuyu and Örnekköy; habitat 10 Hedgerows; habitat 11 Ilica creek banks; habitat 12 Vacant spaces; habitat 13 Karşıyaka railway; habitat 14 Naldöken orchards; habitat 15 Hedgerows of horticultural gardens; habitat 16 Phrygana; habitat 17 Maquis; habitat 18 *Pinus brutia* association; and habitat 19 *Pinus nigra* sub. *pallasiana* association.

Table - 6 : Results of simple linear regression for the response variable of conservation value.

Explanatory variable	Intercept	Coefficient of explanatory variable	r ²	P
Species richness	4.10	2.96	73.1%	0.001
Rarity	7.96	3.33	69.2%	0.001
Area	6.38	2.82	64.5%	0.001
Site age	0.68	3.59	48.7%	0.004
Stratification	4.94	2.60	47.1%	0.005

Table - 7 : Correlation matrix of the variables.

	Rarity	Stratification	Area	Site age	Species richness
Stratification	0.296				
Area	0.630 ^b	0.322			
Site age	0.642 ^b	0.316	0.605 ^a		
Species richness	0.691 ^c	0.678 ^c	0.549 ^a	0.303	
Conservation value	0.832 ^d	0.686 ^c	0.803 ^d	0.698 ^c	0.855 ^d

^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.005$; ^d $P < 0.001$.

and services as the ultimate source and foundation of wealth upon which the urban-rural activities depend are sustained. The identification of urban habitats and the rating of their conservation priority should be accompanied by the monitoring of spatial and temporal patterns of changes in habitats (location, magnitude, direction and rate of changes). This allows management alternatives to

be adapted to dynamic environmental conditions and societal requirements.

With our contemporary understanding of environmental threats, one can see now more clearly that our solutions to environmental threats are tied to our economic development paradigms. Ignorance of our global interdependence, unsustainable population and consumption growth,

distributive injustice of wealth and power, externalities, and lack of coordination for institutional regimes are the major root causes of

the degradation and destruction of the environment (Costanza, 1996). Since cities are the dense concentrations of population, energy-material

Table – 8 : The environmental interactions among major land uses in Karşıyaka.

Environmental Impact	Land uses							
	Housing	Food & chemicals industry	Transportation	Disposal & burning of waste	Burning of fossil fuels	Cropland	Recreation	Nature conservation
Climate								
aerosols	⊙	■	⊙	■	■	●		
air pollution	⊙	■	⊙	■	■	●	●	●
bad odors		■		■	■		●	●
noise			⊙				●	●
GHG emissions		■	■	■	■	■	●	●
Soil								
erosion by water						⊙		●
erosion by wind						⊙		●
soil pollution		■	■	■	■	⊙		●
loss of productive soils	■	■	■			●	●	●
Water								
pollution of surface water	⊙	■		■		⊙		●
pollution of ground water	⊙	⊙		■	■	⊙	●	●
decline in water table						⊙		●
Vegetation								
loss of biodiversity	■		■			⊙	⊙	●
invasive species	■		■			⊙	⊙	●
fragmentation	■		■		■		■	●
Fauna								
loss of biodiversity	■		■			⊙	⊙	●
invasive species	■		■			⊙	⊙	●
fragmentation	■		■		■		■	●
Aesthetics								
visual distortion	⊙		■	■			●	●
homogeneity	⊙	■						

■ = land use and its environmental impact; ● = environmental impact on land use; and ⊙ = land use affecting as well as being affected adversely.

Table – 9 : Amount, density and distribution of open green spaces in Karşıyaka.

Karşıyaka and its districts	Park (m ²)	Density (m ² /person)	Green space (m ²)	Density (m ² /person)
Bayraklı	25,828	0.47		
Bostanlı	149,564	4.16	573,493	2.98
Karşıyaka	126,626	0.54	880,577	6.87
Salhane	-	-	1,305,844	2.04
Toplam	302,018	0.85	131,066	2.54
			2,890,980	2.86

consumption, and waste generation, urban nature conservation strategies should be developed to prevent (1) ecologically incompatible uses of

energy, materials, lands, and species; (2) degradation, fragmentation and destruction of habitats; and (3) reversibly, or irreversibly

Urban nature conservation.

inappropriate conversions among grasslands, forests, croplands, wetlands, and urban-industrial lands. Strengthening institutional conditions for the formulation and implementation of sustainable urban policy and management includes (1) harmonization of the free market with the objectives of participative democracy, social solidarity, and sustainability (2) environmentally adaptive and decentralized approaches in the process of decision- and policy-making; (3) a sound partnership among public and private sectors; (4) a balanced investment in human, natural and man-made resources; and (5) coordination of sectoral policies and institutions.

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Characterization of alkaline phosphatases of some potent phosphate removers.

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Abstract : The alkaline phosphatases of *Vibrio parahaemolyticus*, *Aeromonas hydrophila* and *Micrococcus varians* were subjected to electrophoretic separation to determine the molecular weights of enzyme proteins. The study was emphasized on these bacteria as they were found to remove 92.02%, 89.14% and 87.6% phosphate from the effluents.

Key words : Phosphate, electrophoresis, alkaline phosphatase, molecular weight.

Introduction

Alkaline phosphatase catalyses the hydrolysis of phosphomonoesters to inorganic phosphate and the corresponding alcohol, phenol or sugar. It removes the 5' terminal phosphate and prevents recirculation and dimerization of plasmids used as cloning vectors in biotechnology (Pereira, *et al.*, 1995) and are highly active in the alkaline pH range (Heppel, 1971). Removal of phosphorus by an activated sludge process with an aerobic stage (Barnard, 1975) is expensive and hazardous. Therefore, an attempt was made to remove the phosphate by bacteria and *Vibrio parahaemolyticus*, *Aeromonas hydrophila* and *Micrococcus varians* were found to be the potent phosphate removers from the effluent of Shaw Wallace Gelatins Ltd. These bacteria were found to remove 92.02%, 89.14% and 87.6% of phosphate respectively.

The present study deals with the characterization of alkaline phosphatases of *Vibrio parahaemolyticus*, *Aeromonas hydrophila* and *Micrococcus varians*. Prior to get electrophoresis, alkaline phosphatase assay, effect of pH, temp, incubation period on enzyme activity and total protein concentration was determined.

Materials and Methods

The bacteria used in this study were isolated from treated and untreated effluents of Shaw Wallace Gelatin factory, Jabalpur (M. P.

India) by plating suitable dilutions on to nutrient agar medium. The isolates were screened for phosphatase production using pNpp as substrate (Torrani, 1960) and were identified as *Vibrio parahaemolyticus*, *Aeromonas hydrophila* and *Micrococcus varians* on the basis of morphological and biochemical characteristics according to Bergey's manual of systematic bacteriology (Kreig and Holt, 1984) and PIB Computer Kit (Bryant, 1989) and were maintained on nutrient agar slants. Biological phosphate removal by these phosphatase producers was determined in *In vivo* / *In vitro* condition.

Extracellular alkaline phosphatase assay was carried out by standard methods (Kobori and Taga, 1980). Intracellular alkaline phosphatase was performed following the method of Vasileva and Balasheva (1993).

Characterization of alkaline phosphatase of the above mentioned bacteria was done by studying the effect of pH, temperature and incubation time (min) on the activity and stability of Apase production.

Total protein content was determined by Lowry's method (Lowery *et al.*, 1951).

Gel electrophoresis was carried out by using 8% (w/v) polyacrylamide gel with 0.1% sodium dodecyl sulphate according the Lammeli's method (Lammeli, 1970) to determine the subunits of the enzyme proteins.

Results and Discussion

The optimum pH recorded for alkaline phosphatase activity of *Vibrio parahaemolyticus*, and *Aeromonas hydrophila* was 8.5 and 9.0 in the case of *Micrococcus varians* (Fig 1). When incubated for different time intervals, maximum enzyme activity in *Aeromonas hydrophila* was

found to be at 90 min and in *Vibrio parahaemolyticus* and *Micrococcus varians* it was at 120 min (Table 1). Maximum alkaline phosphatase activity and stability was recorded at 45°C in *Aeromonas hydrophila*, for *Vibrio parahaemolyticus* maximum activity and stability was observed at 30° and 60°C, respectively while

Table – 1 : Effect of Incubation time (mins.) on Alkaline phosphatase activity (IU/ml) over control at 37°C (pH 9.0)

S. No.	Bacteria	Incubation time (min.)		
		30	90	120
1	<i>A. hydrophila</i>	0.040	0.100	0.075
2	<i>V. parahaemolyticus</i>	0.034	0.061	0.099
3	<i>M. varians</i>	0.052	0.150	0.365
	Control	0.012	0.020	0.320

Table – 2 : Effect of Temperature (°C) on Alkaline phosphatase activity for 1 hr. and stability for 2 hr. in IU/ml over control (at pH 9.0).

S. No.	Bacteria	Temperature °C			
		10	30	45	60
Activity					
1	<i>A. hydrophila</i>	0.045	0.085	0.620	0.050
2	<i>V. parahaemolyticus</i>	0.058	0.132	0.166	0.146
3	<i>M. varians</i>	0.260	0.465	0.170	0.410
	Control	0.167	0.258	0.118	0.370
Stability					
1	<i>A. hydrophila</i>	0.240	0.130	0.750	0.57
2	<i>V. parahaemolyticus</i>	0.153	0.147	0.182	0.21
3	<i>M. varians</i>	0.914	0.850	0.045	0.965
	Control	0.870	0.770	0.690	0.940

Table – 3 : Extracellular and Intracellular Protein concentration of bacterial isolates estimated by Lowry's method (Mean values).

S. No.	Bacteria	Code	Extracellular protein content	Intracellular protein content
			(µg/ml)	(µg/ml)
1	<i>Vibrio parahaemolyticus</i>	E	47	121
2	<i>Aeromonas hydrophila</i>	19	58	86
3	<i>Micrococcus varians</i>	30	36	144

in *Micrococcus varians* maximum activity and stability was observed at 30° and 60°C, respectively (Table 2).

Maximum extracellular alkaline phosphatase was observed in *Vibrio parahaemolyticus* (0.051 IU/ml) on 12th day of incubation while minimum was observed in

Aeromonas hydrophila and *Vibrio parahaemolyticus* (0.005 IU/ml) on 2nd day of incubation. In *Aeromonas hydrophila* maximum intracellular alkaline phosphatase activity was noticed on 12th day of incubation (0.083 IU/ml) and minimum activity was found on 2nd day of incubation (0.036 IU/ml) (Fig 2). The phosphatase activity and poly (P) accumulation occurs during

Characterization of alkaline phosphatases.

exponential/logarithmic phase and also, Apose production is regulated by phosphate starvation as reported in the bacteria (Kulev and Vagabov, 1983).

Prior to the gel electrophoresis, total extra and intracellular protein concentration determined. Maximum extracellular protein content was found to be in *Aeromonas hydrophila* (58µg/ml) while

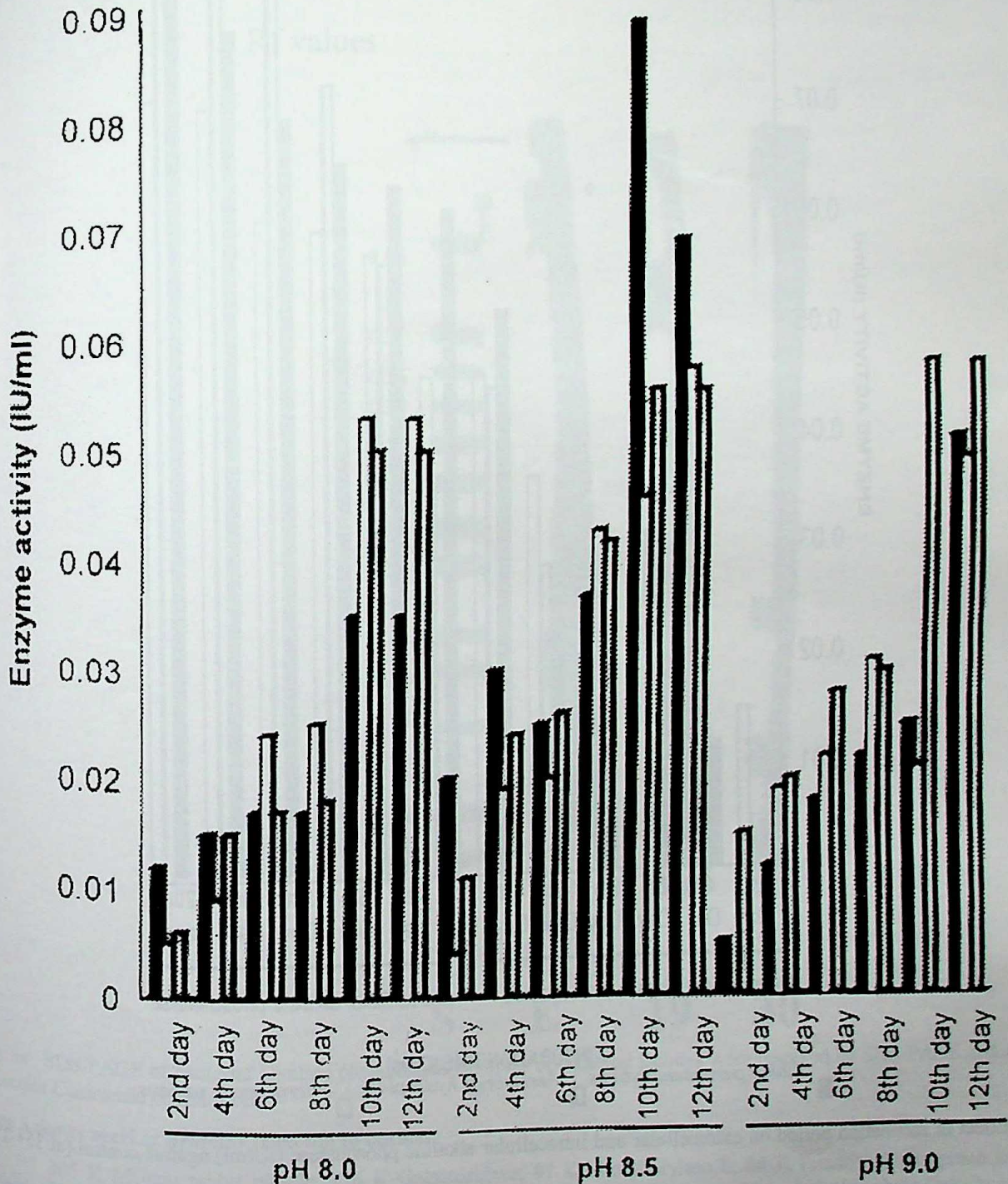


Fig. 1 : Effect of pH 8.0, 8.5, and 9.0 on optimization of extracellular alkaline phosphatase activity (IU/ml) against control (at 35±2°C).

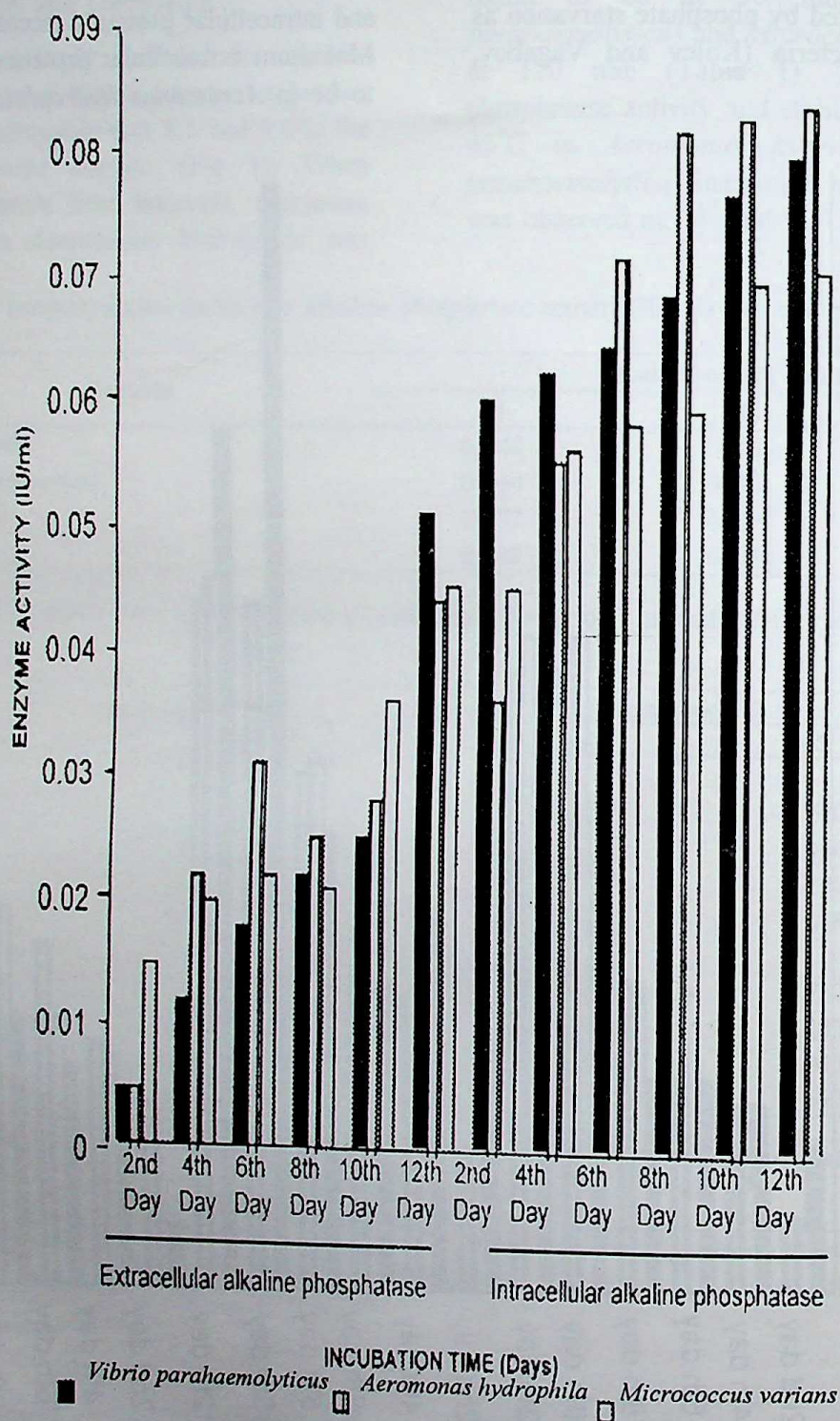


Fig. 2 : Effect of incubation period on extracellular and intracellular alkaline phosphatase (IU/ml) against control (at $35 \pm 2^\circ\text{C}$, pH 9.0).

maximum intracellular protein content was observed in *Micrococcus varians* (144 $\mu\text{g/ml}$) (Table 3).

The alkaline phosphatases of the above mentioned microorganisms were analyzed for their molecular weights by performing electrophoresis using SDS-PAGE.

Most of the alkaline phosphatases reported in the literature have molecular weights in excess of 80,000 da and most appear to consist of subunits with molecular weight of at least 40,000 da (McComb *et al.*, 1979). While in our studies

alkaline phosphatase of *Vibrio parahaemolyticus* had a value of 0.773 corresponding to the molecular wt. of 18,500 da. *Micrococcus varians* produced three defined band with respective migration distance of 0.53, 0.76 and 0.84 having

Rf values

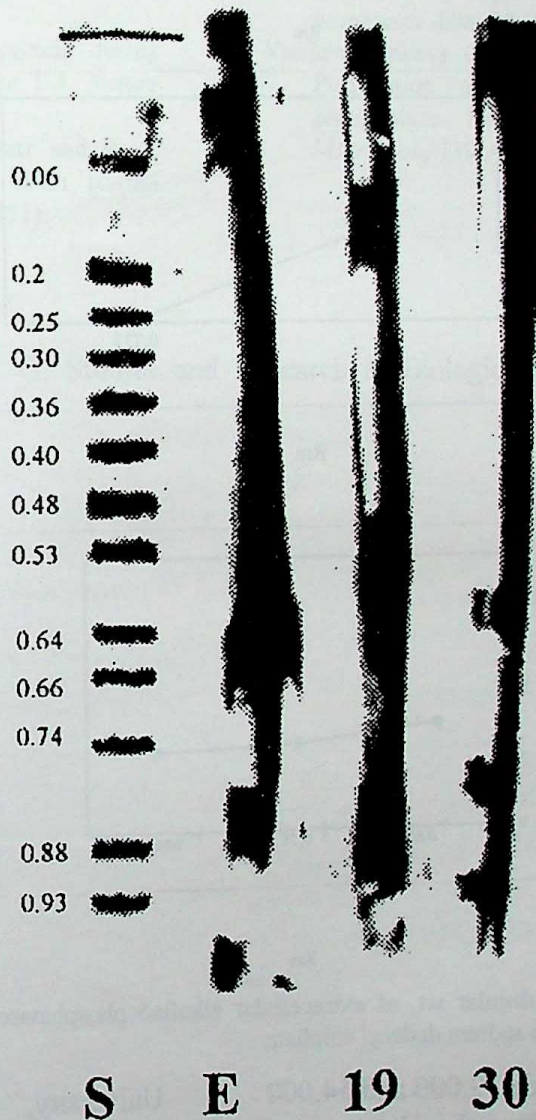


Fig. 3a : SDS-PAGE of bacterial alkaline phosphatase. Samples of bacterial isolates were resolved by SDS-PAGE and stained with molecular Coomassie brilliant blue.

Lane S shows marker proteins from top to bottom :

205 K Myosin rabbit muscle; 116 K Galactosidase; 97 K Phosphorylase b; 84 K Fructose 6 phosphate kinase; 66 K Bovine serum albumin; 55 K Glutamine Dehydrogenase; 45 K Ovalbumin; 36 K Glyceraldehyde-3 phosphate Dehydrogenase; 29 K Carbonic Anhydrase; 24 Trypsinogen; 20 K Trypsin Inhibitor; 142 K a-lactalbumin; 6.5 K Aprotinin Bovine.

E	=	<i>Vibrio parahaemolyticus</i>
19	=	<i>Aeromonas hydrophila</i>
30	=	<i>Micrococcus varians</i>

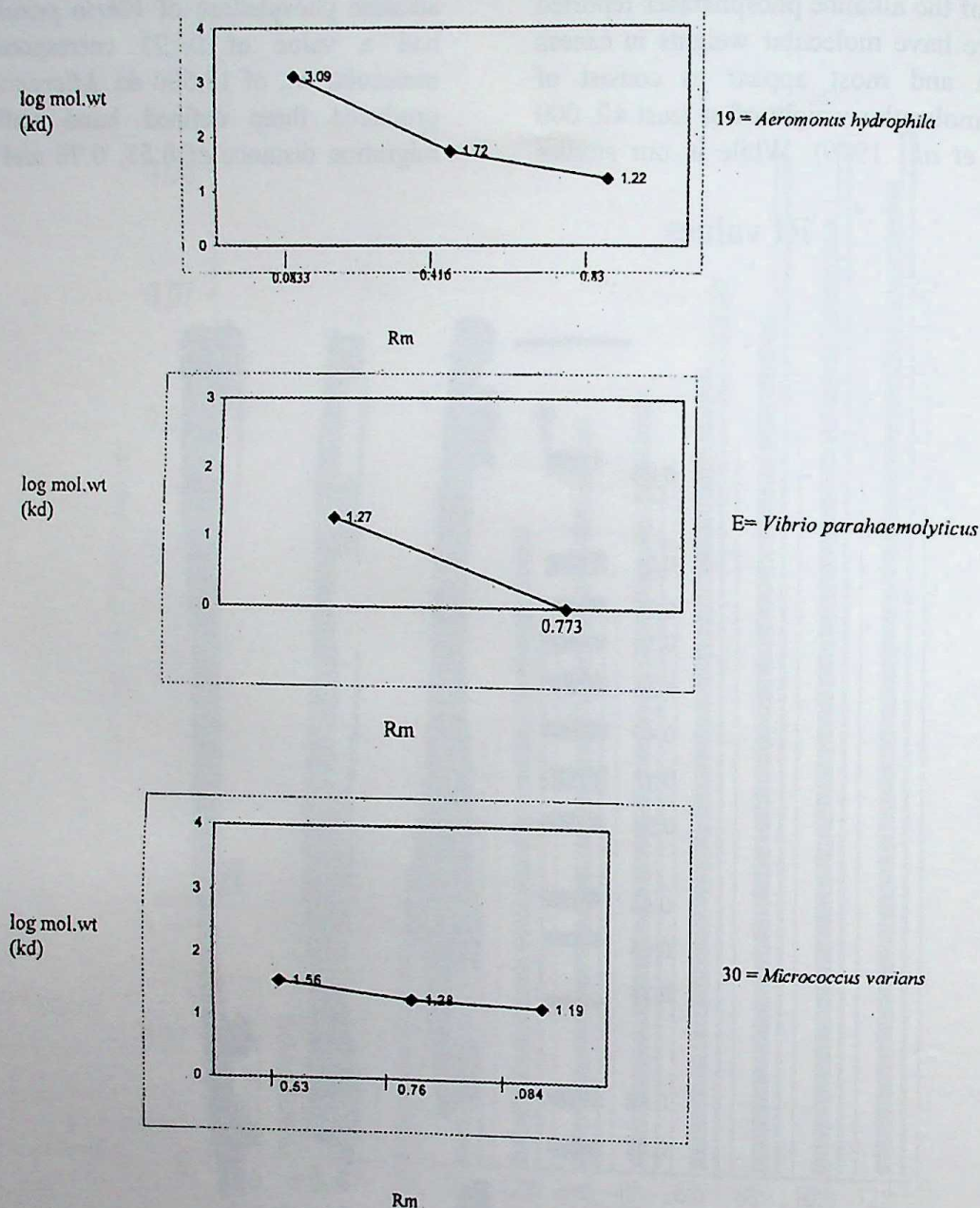


Fig. 3b : Rm values and log molecular wt. of extracellular alkaline phosphatase of bacterial isolates as determined by gel electrophoresis in presence of 0.1% sodium dodecyl sulphate.

molecular weights of 36,000 da. 19,000 and 54,000 da. Extra-cellular alkaline phosphatase of *Aeromonas hydrophila* produced three distinct bands corresponding to the molecular weights of 1, 220, 000, 52, 000 and 16,500 da with relative mobilities of 0.083, 0.416 and 0.083 (Fig 3a, 3b).

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Bioaccumulation of fenvalerate technical grade in different organs of the frog *Haplobatrachus tigerinus* (Daudin).

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Abstract : Bioaccumulation studies of fenvalerate were conducted on Indian bullfrog *Haplobatrachus tigerinus* (Daudin) after exposure to sublethal intraperitoneal dose of technical grade fenvalerate (1/3 LD₅₀ i.e. 116.66 µg/kg body weight) at 3, 6, 12, 24, 48 and 72 hours schedule. The tissues viz., muscle, liver, kidney, testis, brain, and whole body accumulation was analysed for residue estimations. In all the tissues, analysed maximum residue was recovered in the initial stages of exposure (3 and 6 hours). However, in brain the residues remained upto 72 hours. In the whole body, analysis after 3 hours of exposure 78.65% residue was recovered and by the time 72 hours passed only, 9.4% residue was recovered. The decline in residue levels along with the period of exposure indicates the fast acting nature of fenvalerate and metabolites.

Key words : Gas liquid chromatography, fenvalerate, *Haplobatrachus tigerinus*.

Introduction

Pyrethroid insecticides are an important group of chemicals that are constantly gaining popularity. Fenvalerate (RS) (α-cyano-3-phenoxybenzyl (RS)-2-(4-chloro-phenyl)-3methylbuterate is an effective synthetic pyrethroid insecticide possessing an excellent insecticidal activity and also environmental stability (Bradbury *et al.*, 1982). Fenvalerate contains two asymmetric carbon atoms at the acid and alcoholic moieties. Generally, crustaceans, molluscs and fish are more sensitive to fenvalerate followed by amphibians, reptiles, birds and mammals (Mulla *et al.*, 1978; Linden *et al.*, 1979; Coats and O'Donnell Jaffery, 1979; McLeese *et al.*, 1980). Bioaccumulation factors and mortality with chlorinated compounds were also discussed extensively (Kuehl *et al.*, 1995).

The wide spread occurrence of residues or metabolites of pesticide was reported to be related to its persistence in the environment coupled with heavy and prolonged use (Crockett *et al.*, 1974). Fenvalerate is gaining wide acceptance in agricultural usage and is destined for increased use against agricultural, poultry, dairy and household pests. Pyrethroids can be bioaccumulated by

individual organisms during acute or chronic exposure to sublethal concentrations (Smith and Stratton, 1986). Bioaccumulation can be considered a sublethal effect of pesticides, but usually the organism evidences little impairment of normal activity or function.

Chromatographic analysis of fenvalerate in the animal material was studied by FAO/WHO (1985). However, their persistence in aquatic ecosystems may be sufficient to cause environmental impact from direct field application because of their extremely high toxicity and fast acting nature (Spehar *et al.*, 1982). Much work carried out on the toxicity evaluation, residue analysis, kinetics, metabolism of fenvalerate on crustaceans, molluscs, fish, birds, mammals, soil and water. A national level study of chemical residues in various fish was also studied extensively (Kuehl *et al.*, 1995; Marquis *et al.*, 1994; Kulshrestha *et al.*, 1995). As there exist very little information on frogs an attempt has been made to study the residue levels of fenvalerate technical grade in the selected tissue of edible frog *Haplobatrachus tigerinus* (Daudin) exposed to sublethal concentrations for 3, 6, 12, 24, 48 and 72 hours by Gas-liquid chromatography.

Materials and Methods

Adult male frogs of *Haplobatrachus tigrinus* with a weight range of 100 ± 10 grams with an average snout vet length of 10.61 ± 0.2 cm were collected in and around Vijayawada and Guntur, Andhra Pradesh, India and acclimatised to the laboratory conditions (25°C to 30°C) for one week in cement wells covered with wire mesh. Fenvalerate (RS), a synthetic pyrethroid compound, technical grade 93.7% (w/v) was supplied by M/s. Gujarat Insecticides Limited, Ankleswar, Gujarat State, India. The dose was prepared by dissolving technical grade fenvalerate in acetone. Each frog was weighed and treated with a single dose of the pyrethroid fenvalerate ($1/3 \text{ LD}_{50}$ i.e. $116.66 \mu\text{g/kg}$) intraperitoneally. The control frogs were treated with equal amount of acetone without the toxicant.

Muscle, liver, kidney, testis and brain of sacrificed immediately after the test period frogs were removed, weighed and stored for ($< 4^\circ\text{C}$) subsequent analysis. (Saleh *et al.*, 1986) method with minor modifications was followed for the extraction of residues from the tissue. Extracts were cleaned up on silica gel columns covered with

a layer of anhydrous sodium sulfate and packed with hexane (Goughan *et al.*, 1978).

Gas liquid chromatography was carried out on Hewlett-Packard Model 5840, a gas chromatograph with FID (Flame Ionization Detector) and glass coiled column (6 feet x $\frac{1}{4}$ inch internal diameter) packed with 10% SE 30 on 80-100 mesh chromosorb WAW. The column temperature was 280°C injector and detector temperatures were 300°C respectively. Nitrogen was used as the carrier gas at 50 ml/min. 5 μl of each sample was analysed thrice with and without adding known amounts (100 ng/gr of sample) of fenvalerate as an internal standard.¹

Results and Discussion

The calculated values for the fenvalerate residue in different tissue viz., muscle, liver, kidney, testis, brain and whole body at different periods of exposure were presented in Fig.1.

Analysis of tissue of frog following intraperitoneal administration of fenvalerate revealed that fenvalerate was present in all the samples examined. In muscle and the highest residue was

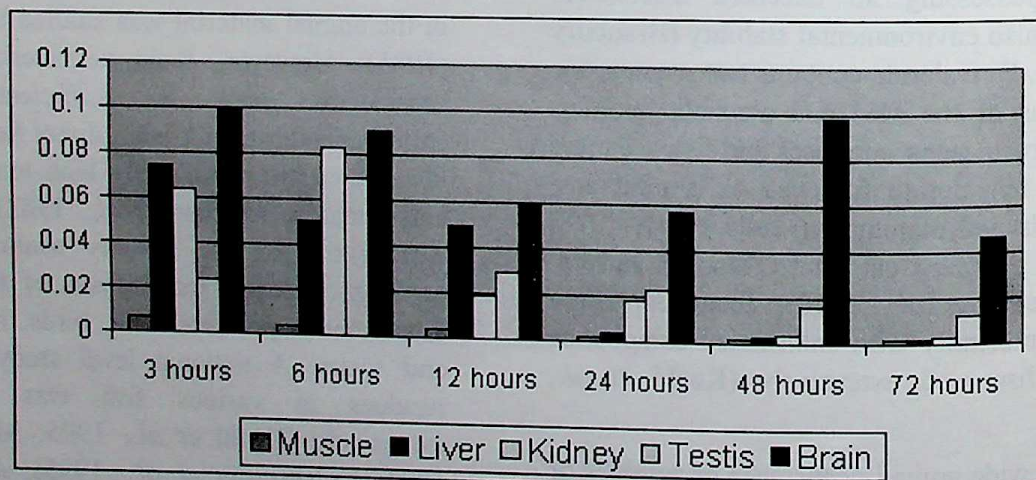


Fig. 1 : Residue levels of synthetic pyrethroid fenvalerate in different tissue of *Haplobatrachus tigrinus* (Daudin) at different times after injection of sublethal dose of fenvalerate treated intraperitoneally. The values are expressed as $\mu\text{g/gram}$ wet weight.

recovered at 3 hours and decreased in the subsequent periods of exposure. In kidney and testis the residue, maximum recovery was at 6 hours and decreased in the subsequent times. In

brain, maximum residue was noticed at 3 hours and decreased upto 24 hours. At 48 hours time, the residue level increased and by 72 hours decreased. In brain tissue from 6 to 72 hours of exposure, the

Bioaccumulation of fenvalerate in different organs of the frog.

residue of maximum fenvalerate was at and is in correlation with the results of (Ghousia Begum *et al.*, 1994) in *Clarias batrachus*.

The recovery of fenvalerate residue from the whole body analysis was shown in Fig.2. At three hours of exposure, the residue level was 92.6

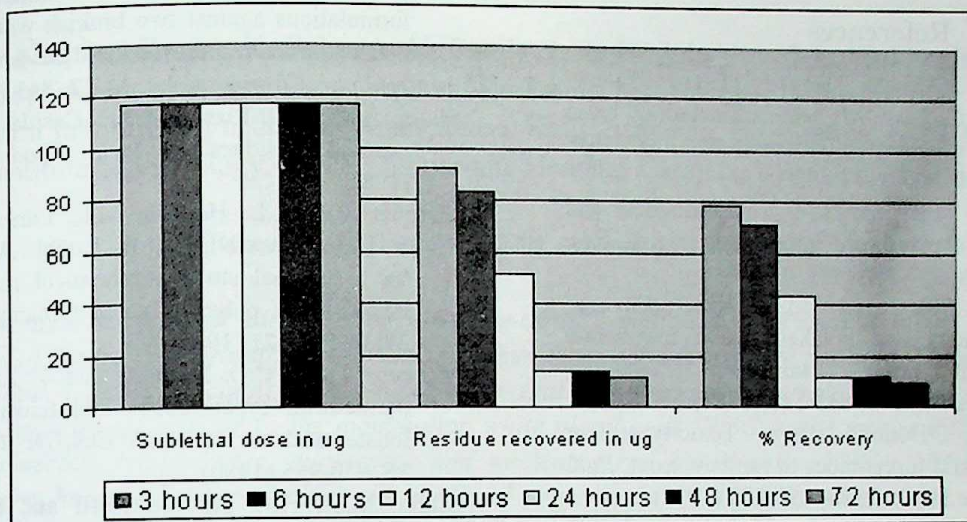


Fig. 2 : Residues of fenvalerate technical grade recovered in the whole body analysis of the frog *Haplobatrachus tigrinus* (Daudin) after a single intraperitoneal dose.

μg with a recovery percent of 78.65. At 6 hours, the percent recovery was 70.94, at 12 hours 43.58 and at 24 and 48 hours 11.11 and 11.96 respectively. After 72 hours, the residue recovery was only 9.4%.

Pesticide fenvalerate residue depends on its solubility, interaction, chemical structure, condition of the animal, variations in metabolic pattern and habitat (Johnson, 1968). As the dose was intraperitoneal and given above the pelvis region, near to testis, the residue level was high at 3 hours. Pyrethroid residues decrease less rapidly in fat and seem to be in consistent in the case of brain (Bradbury and Coats, 1989). In the present study also, similar result was observed. Higher tissue concentration of pesticide is generally associated with lipid deposits of the body, which is consistent with the lipophilicity of pyrethroids (Saleh *et al.*, 1986). Pyrethroid levels initially attained in fat and brain are probably inversely related to their relative rates of metabolism in liver (Marei *et al.*, 1982). Bradbury *et al.*, (1985) observed highest residue in bile followed by fat in trout. Liver and kidney being highly perfused organs showed peak concentration of fenvalerate

residue at 3 hours and 6 hours respectively which derives support from the study of Bradbury and Coats (1989).

The study of fenvalerate deposition in both Japanese Quail (Mumtaz and Menzer, 1986) and bobwhite quail (Bradbury and Coats, 1982) at the rate of 1.5 mg/kg/day for 14 days, where 75% of the initial pyrethroid was excreted within 24 hours and 85 to 90% excretion occurred at 48 hours, which is similar in the present observation.

In conclusion, since pesticide residues are known to accumulate in the lipid tissues of non-target organisms, which are like fish, frog and other animals and are known to be transferred via food chain to the human bodies, the grave risk to the health of the people who consume these organisms seems to be considerable. The need to protect the fast declining populations like frogs, which are natural pest controllers from undue exposure to the insecticides, cannot be ignored and also the need to protect the people who consume the fish and frogs from undue exposure to the pesticide residues via food chain cannot be overemphasized. Fenvalerate residues will also

show impact on reproductive impairment of the commercially important organisms like fish and frog.

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Ultra-structural observations on the lymphoid organs of the freshwater catfish, *Clarias batrachus* (Linnaeus).

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Abstract : Light microscopic and ultra-structural studies of the lymphoid tissues such as blood immunocytes, spleen and pronephros of the freshwater catfish, *Clarias batrachus*, were carried out. The peripheral blood showed nucleated erythrocytes, total leucocytic count (TLC) more than that observed in mammalian blood and leucocytes with morphological appearance similar to the mammalian white blood cells (WBCs). The spleen and pronephros showed presence of numerous lymphocytes, monocytes and nucleated red blood cells (RBCs) along with hemosiderin-containing macrophages. The morphology of lymphoid organs of the catfish has been discussed in light of the evolution of the immune system in this class of vertebrates.

Key words : Lymphoid organs, Erythrocytes, Lymphocytes, Monocytes, *Clarias batrachus*.

Introduction

The immune system covers a wide spectrum from a simple to a complex apparatus, which develops during the phylogenetic scale of evolution. Its primary object is to prevent the life-threatening invasion of indigenous as well as exogenous microorganisms (Paterson, 1986). In lower vertebrates such as fish, which are constantly in contact with water-borne microorganisms, the immune organs are surprisingly simple, less organized and poorly developed (Manning and Turner, 1976; Roberts, 1989). They consist mostly of thymus (Grace *et al.*, 1980), spleen (van Loon *et al.*, 1980), pronephros (Rijkers *et al.*, 1980) and gut-associated lymphoid tissue (GALT) (Roitt *et al.*, 1986).

Though Sailenderi and Muthukarruppan (1975) have studied ontogeny of the lymphoid organs in cichlid fish (*Tilapia mossambicus*) by histological methods, we describe ultra-structural observations on the lymphoid organs of the freshwater catfish, *Clarias batrachus* in the present communication.

Materials and Methods

Adult catfish, *Clarias batrachus* (Linnaeus) (both sexes; average body weight 77.7 ± 33.7 gm), were obtained afresh and kept in aquarium equipped with an aerator and thermostat. The temperature of the aquarium water was maintained at $22 \pm 2^\circ\text{C}$. The fish were anaesthetized with MS222. The head kidney (pronephros) and spleen were dissected out and processed for histological as well as ultra-structural studies. Blood was collected from caudal vein of the fish in heparinized tubes.

Light Microscopy : Blood smears on glass slides were stained with Giemsa's stain. Spleen and pronephros were fixed in 10% neutral buffered formalin, cut into small pieces, dehydrated with a series of graded alcohol, cleared in xylene and embedded in paraffin wax at 60°C . $5\ \mu$ thick sections were cut from these blocks and stained with hematoxylin and eosin (H&E).

Electron Microscopy : Heparinized blood from *Clarias batrachus* was centrifuged at 3000 rpm for 15 minutes. The plasma layer was pipetted out and replaced with 2.5% glutaraldehyde. On

keeping for 1/2 to 1 hour at 40°C, the buffy coat was converted into a semisolid button, which could be removed by a platinum wire and cut into pieces so that vertical sections of buffy coat were obtained. The pronephros and spleen were also collected from live catfish in 2.5% glutaraldehyde and processed for electron microscopy (Meek, 1977). The tissues were fixed in glutaraldehyde overnight at 4°C. Thereafter, they were washed in sucrose cocodylate buffer (pH 7.2) and distilled water and post-fixed in osmium tetroxide. This was followed by dehydration with graded alcohol, cleaning and infiltration in graded mixtures of propylene oxide and epon (3 : 1; 1 : 1; 1 : 3) and final embedding in Epon812. The polymerized blocks were then trimmed in an ultramicrotome, semi-thin sections (1 μ thick) were cut, stained with 1% toluidine blue in 1% borax solution and viewed

under a light microscope for initial evaluation. Blocks with specific areas were then chosen and ultrathin sections were cut with a glass knife. These sections were then stained with uranyl acetate and lead citrate for electron microscopy.

Results and Discussion

The average body and spleen weights of 7 normal *Clarias batrachus* were 77.7 ± 36.7 gm and 0.1 ± 0.05 gm, respectively. Thus, the ratio of spleen weight to body weight was 0.0013 ± 0.0004 (0.0009-0.0021). The pronephros was located in the body cavity, outside the peritoneum and ventral to the vertebral column. Its size varied from 12 to 17 mm in length and the colour was dark brown. Its average weight was 146 ± 51.1 mg (84-203 mg).

Table 1 : Differential leucocyte counts in peripheral blood of normal catfish, *Clarias batrachus*.

Type of blood leucocytes	Number of cells/mm ³	Percentage (%)
Total leucocyte cells	1.2685×10^6	
Lymphocyte	0.647×10^6	51%
Granulocyte	0.317×10^6	25%
Monocyte	0.101×10^6	8%
Plasma cell	0.05×10^6	4%
Basophil	0.038×10^6	3%
Acidophil	0.038×10^6	3%
Vacuolated cell	Not found	---

Light and Electron Microscopic Observations

Blood : The mean total blood leucocyte count (TLC) of normal *Clarias batrachus* was 1.2685×10^6 per cubic mm. Table 1 shows the differential leucocyte count in the peripheral blood. The peripheral smear showed red blood cells with uniformly oval cell outlines and centrally located nuclei. The leucocytes could be classified into lymphocytes, polymorphonuclear leucocytes, monocytes and eosinophilic leucocytes. Ultra-structural examination confirmed the presence of granules similar in appearance to neutrophilic and eosinophilic granules as seen in the mammalian blood (Fig.1).

Pronephros : Histological study of normal pronephros revealed the presence of sinusoids with nucleated erythrocytes, separated by cords consisting of histiocytes and lymphocytes. Histiocytes with collection of hemosiderin pigments in their cytoplasm were also seen. Electron microscopy of pronephros showed monocytes with characteristic kidney-shaped nuclei and abundant cytoplasm containing lysosomal granules (Fig.2). Nucleated erythrocytes were seen in between the monocytic cells (Fig.3).

Spleen : The normal spleen of *Clarias batrachus* showed a great similarity to that of higher mammals. It consisted of red and white pulps as

well as sinusoids which were separated by septae containing blood vessels and lymphocytic cells. However, unlike higher animals, the white pulp was not clearly demarcated. Hemosiderin pigments were found. Ultra-structural examination of the spleen of *Clarias batrachus* showed cells with smooth external contours and without any cytoplasmic projections. Monocytes had moderate cytoplasm, few filipodia and few lysosomes (Fig.4). The nucleus of the lymphocyte was large and was surrounded by a thin layer of cytoplasm containing few organelles. The cells in the sinusoidal space were lined by cytoplasmic extensions of reticular cells (Fig.5).

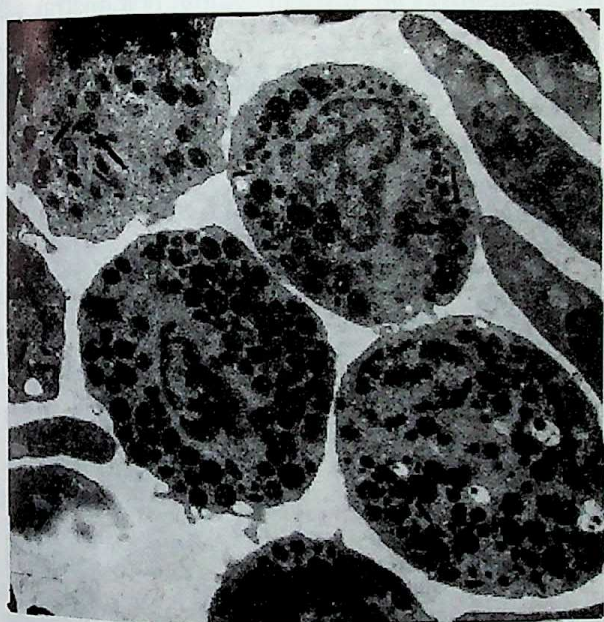


Fig. 1 : Electron micrograph of the buffy coat preparation from *Clarias batrachus* showing three granular lymphoid cells having typical primary and secondary granules of neutrophilic leucocytes, however, the nuclei do not show any multilobation. Cytoplasm of the other cell contains granules (arrow) with central dense core indicating eosinophilic leucocytes. X 8,000.

Evolutionary study of lymphoid tissues among vertebrates suggests that thymus and spleen appeared phylogenetically first in the elasmobranchs. In teleosts, thymus, spleen, kidney and perhaps gut-associated lymphoid tissue (GALT) are well-developed while bone marrow and lymph nodes are absent (Roberts, 1989). The

fishes also mount strong MIR (mixed lymphocyte reaction) and allograft rejection reactions (Roitt *et al.*, 1986). The absence of thymus in *Clarias batrachus* as seen in the present study could be due to its involution during sexual maturity (Manning and Turner, 1976; Roberts, 1989).

In *Clarias batrachus*, the erythrocytes were nucleated and exhibited different shapes from oval to round (Fig.2). The leucocytes were either granular or agranular, however, the number of agranular cells were more than granular cells. Granulocytes in the blood of *Clarias batrachus* included acidophils, basophils and neutrophils

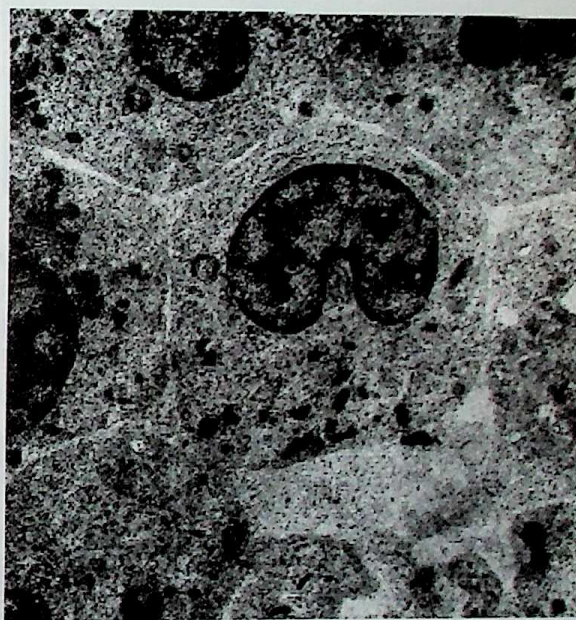


Fig. 2 : Electron micrograph of a section of pronephros from *Clarias batrachus* displaying a monocytoic cell with characteristic kidney-shaped nucleus and abundant cytoplasm with several lysosomal granules. X 8,000.

whereas agranulocytes were mainly lymphocytes and monocytes (Table 1). The number of blood lymphocytes in the catfish was about one thousand times more than that recorded in the human blood. Unlike mammals, lymphoid tissues were not well-organized and no lymph nodes were present in *Clarias batrachus*. Thus, the large number of circulating lymphocytes in the catfish might be necessary for protection against microorganisms. The pronephros of *Clarias batrachus* was richly

supplied with lymphoid cells, granulocytes, phagocytes and antibody-producing cells (Fig.5).



Fig. 3 : A section of pronephros of *Clarias batrachus* exhibiting nucleated RBC (arrow) in between the group of monocytooid cells. The mononucleoid cells depict smooth contour and few cytoplasmic organelles. X 11,000.

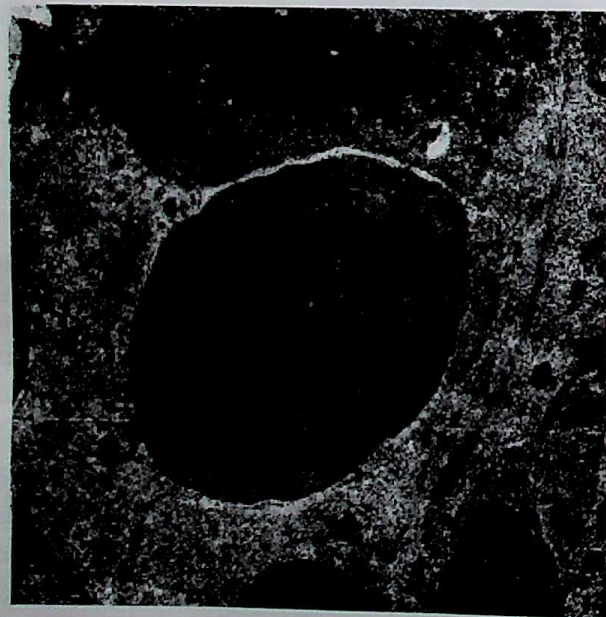


Fig. 4 : An electron micrograph from spleen of *Clarias batrachus* showing monocytooid cells with kidney-shaped nucleus, few organelles and cytoplasm having smooth external contour. X 11,000.

Sailendri and Muthukkaruppan (1975) reported that the head kidney of *Tilapia mossambicus* was a peripheral lymphoid organ as evident by the presence of lymphoid follicles and cellular changes due to immunization. The present study also gain support from the report of Ellis (1976) who has shown that the pronephros and opisthonephros of fish contain hemopoietic tissue, and is rich in lymphoid cells and granulocytes. Phagocytes and plasma cells have also been reported to be present in fish kidney (Rijkers *et al.*, 1980). Earlier, Ellis (1977) and Agius (1979) described that pigment-bearing cells were mostly found in fish kidney. These pigments are mainly melanin, lipofuscin and hemosiderin. The present study demonstrates the



Fig. 5 : Electron micrograph from spleen of *Clarias batrachus* exhibiting a nucleated RBC (arrow) along with two small lymphocytes. X 11,000.

aggregation of hemosiderin pigments in catfish pronephros and spleen. Similar types of melanomacrophage centres with aggregation of pigment cells were also found in the teleostean kidney (Agius, 1980). The histology of spleen of *Clarias batrachus* showed hemosiderin pigments were more in spleen tissue in comparison to that of head kidney. Further, Agius (1979) reported that the iron content was more characteristic of pigment cells in the spleen. Sailendri and Muthukkaruppan

(1975) found the appearance of a maximum number of plaque-forming cells in the spleen and head kidney of immunized *Tilapia mossambicus*. The ultrathin sections of pronephros and spleen of the freshwater catfish showed monocytoïd cells having kidney-shaped nucleus with few organelles. The cytoplasm had smooth external contour.

The Buffy coat from blood of *Clarias batrachus* showed large granular lymphoid cells, perhaps NK (natural killer) cells, acidophils with boat-shaped granules, polymorphonuclear leucocytes and lymphoid cells with filipodia. This preliminary study warrants further investigation on the various receptors on the above cells such as Fc, C3a, C5a, CRI and CR3. Studies on peroxidase, acid phosphatase and alkaline phosphatase reactivity of these cells are also necessary.

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The effect of lead bioaccumulation on haem biosynthetic enzymes in fish.

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Abstract : The bioaccumulations of lead in the liver and hepatic microsomes of fish after 1, 3, 7, 14, 28, and 45 days exposure were studied. In addition, the relationship between the bioaccumulated lead in both hepatic microsomes and the liver and their haem biosynthetic enzymes were studied. Lead toxicity was shown to result in a depression of the microsomal mixed function oxidase system, as assessed by a decrease in hepatic microsomal cytochrome P-450 and b5 content and by a decrease in the activity of the enzymes aniline hydroxylase and aminopyrine demethylase. Lead had a more marked effect on cytochrome P-450 than b5. The activity of the rate-limiting enzyme of haem biosynthesis, δ -aminolevulinic acid synthase, was inversely correlated with the microsomal cytochrome P-450 content. The activity of the haem biosynthetic enzymes δ -aminolevulinic acid dehydratase, coproporphyrinogen oxidase and ferrochelatase were decreased by increasing lead pretreatment. The activity of the haem catabolic enzyme, haem oxygenase, was increased by concentration and length of time to lead exposure.

Key words : Biosynthesis of haem enzyme, Hepatic cytochrome, Delta aminolevulinic, Lead toxicity.

Introduction

Heavy metal toxicity results in biochemical changes leading to systemic organ malfunctions. It has long been recognized that haem synthesis in man is severely affected by increased body lead burden (Al-Ayed, 1996). This inhibition of haem production is well known to result in decreased levels of circulating haemoglobin in lead-exposed individuals. Reduction in blood hemoglobin leads to an overall cardio-respiratory insufficiency (Haffor, 1987). The possibility of altered drug metabolism due to inhibition of synthesis of the microsomal haemoprotein cytochrome P-450 has been the subject of a number of studies both in man and animals.

Recent reports (Alvares *et al.*, 1975; Meredith *et al.*, 1977; Taylor and Russo, 1999) of human studies have shown that in acute lead intoxication in man there is a depression in the elimination rates of phenazone (antipyrine), a drug metabolized by the liver cytochrome P-450-dependent microsomal mixed function oxidase system. This interpretation was supported by the

finding of decreased half-lives and increased clearance of the drug following chelation therapy. Chelation therapy was also shown (Meredith *et al.*, 1977) to result in a fall in blood lead levels and a rise in hemoglobin and erythrocyte δ -aminolevulinic acid (ALA) dehydratase activity, an intermediate enzyme of the haem biosynthetic pathway.

The literature contains a number of studies regarding measurement of toxic effects of heavy metals. However lead toxicity studies that deal with microsomal oxygenase activity have not been carefully studied in aquatic organisms

Preliminary studies by Ribeiro (1970) on the effects of arsenic, beryllium, lead and mercury on mouse liver drug metabolizing enzymes indicated that pretreatment of mice with lead nitrate to give liver levels of 10^{-4} and 10^{-6} M did not alter hexobarbitone sleeping times. When lead was added to control microsomes at concentrations between 10^{-3} and 10^{-5} M, no inhibition of hexobarbitone oxidase was noted. In contrast, the results of Alvares *et al.* (1972) and Scoppa *et al.* (1973) suggest that lead has a significant effect on

microsomal drug metabolism. Alvares *et al.* (1972) found a 40 to 50% decrease in metabolism of drugs by hydroxylation and demethylation and a significant decrease in cytochrome P-450 levels and an increase in cytochrome P-450 levels and an increase in hexobarbitone sleeping times, 24 h after the intravenous injections of lead chloride. From the results of their studies, the authors considered that lead exerts its effect on drug metabolism via an inhibition of haem synthesis.

Scoppa *et al.* (1973) attempted to verify this hypothesis by correlating the inhibition of blood and liver ALA dehydratase by lead with drug metabolism. They were able to demonstrate lowered activity of this enzyme and a depression in cytochrome P-450 levels and an associated depression of in vitro activities of the microsomal mixed function oxidase system.

This study aimed to investigate the effects of lead toxicity on microsomal cytochrome P-450 and the associated mixed function oxidase system, along with the effects of lead on haem metabolism in fish. The latter objectives include the measurement of the activities of six of the enzymes of haem biosynthesis including the rate-limiting enzyme ALA synthase and the measurement of haem oxygenase activity. This latter catabolic haem enzyme has been shown activated in hepatic microsomal preparations of lead-poisoned fish (Deliville, 1999; Maines and Kappas, 1976a).

Materials and Methods

Fish (Crap, *Cyprinus carpio*, range of body weigh 46 to 60 gm) were purchased from Fish Network Station-Derab, South of Riyadh City, Saudi Arabia. Fish were divided into a control and three experimental groups of 5 fish each. Fish were randomly assigned to each group. During the experiments, fish were kept in glass aquaria (60 × 30 × 20 cm) containing 10 liter chloride free tap water with PH 7.0-7.4; total hardness = 141 223 mg/l (as CaCO₃); dissolved oxygen = 7.5-11.5 mg/l. The water temperature was kept at 20 ± 2°C. During the experiment fish were fed once a day with rifran, special aquaria

fish mixture. Water was changed every 24 hours, followed by addition of fresh lead solution.

Toxicity testing : Fish were exposed to different concentrations of lead for 1, 7, 14, 28, and 45 days. Lethal concentration inducing 50% mortality (LC₅₀) was calculated by Wilcoxon method (1949). At the end of each exposure period, blood samples were taken before the fish were sacrificed. Livers were collected, fresh, weigh, and prepared for biochemical analysis or for histo-chemical examinations.

Preparation for microsomal analysis : The fish were sacrificed and their livers perfused *in situ* with ice-cold saline; all further procedures were carried out at 4°C. The livers were quickly removed, blotted and weighed and 25% (w/v) homogenates in 0.15 M KCl were prepared with a Potter-Elvehjem homogenizer. The homogenate was spun for 10 min at 900 g and supernatant produced spun at 20,000 g for 15 minutes. The microsome containing supernatant was spun at 105,000 g. The microsomal pellet produced was washed by resuspending it in 0.15 M KCl, and recentrifuging for a further 60 min at 105,000 g. The resulting washed microsomal pellet was suspended in 0.15 M KCl to give a concentration of 6 mg protein/ml.

Microsomal analysis : The quantity of microsomal cytochrome P-450 and b₅ were determined according to the method of Omura and Sato (1964). The amount of cytochrome P-450 was expressed as nmol of P-450/ mg microsomal protein, while the amount of cytochrome b₅ was expressed as ΔE 423nm-410 nm/ mg microsomal protein.

The microsomal aniline hydroxylase activity was determined by a method described by Schenkman *et al.* (1967). The activity was expressed as nmol p-amino phenol produced per mg microsomal protein in 1 h (nmol p-aminophenol/ mg microsomal protein/ h) at 37°C.

Microsomal aminopyrine demethylase activity was determined by the method of Cochlin

Effect of lead bioaccumulation on fish.

and Axelrod (1959). The activity was expressed as nmol of formaldehyde formed per mg microsomal protein in 1 h (nmol formaldehyde/ mg microsomal protein/ h) at 37°C.

Microsomal haem oxygenase activity was determined by the method of Maines and Kappas (1975). The activity was expressed as nmol bilirubin formed per mg microsomal protein in 1 h (nmol bilirubin/ mg microsomal protein/ h) at 37°C.

The activities of haem biosynthetic enzymes ALA synthase, ALA dehydratase, prophobilinogen (PBG) deaminase, uroporphyrinogen (URO) decarboxylase, coproporphyrinogen (COPRO) oxidase and ferrochelatase were determined by the method of Brodie *et al.* (1977). ALA synthase activity was determined in 50% (w/v) homogenates in 0.15 M KCl from whole liver. In the 5 fish, in each group, the remaining four-haem biosynthetic enzyme activities were determined in 33% (w/v) homogenates in 0.15 M KCl from pooled liver samples, each sample being matched from two fishes with the corresponding control group. The activities were expressed as : ALA synthase, nmol ALA formed per g homogenate protein in 1 h at 37°C; ALA dehydratase, μ mol ALA utilized Per g homogenate protein in 1 h at 37°C; prophobilinogen deaminase, nmol URO formed per g homogenate protein in 1 h at 37°C; uroporphyrinogen decarboxylase, nmol COPRO formed per g homogenate protein in 1 h at 37°C; Coproporphyrinogen oxidase, nmol CPRO formed per g homogenate protein in 1 h at 37°C; ferrochelatase percent ct/ min of ^{59}Fe incorporated into haem per g protein in 1 hour.

Lead levels were determined in microsomal suspensions and liver homogenates by the inductive coupling plasma (ICP) according to method of Protasowicki (1995). Protein was determined by the method of Lowry *et al.* (1951).

Statistical analysis : Multivariate Analysis of variance (MANOVA), was utilized for testing the overall effects of the independent variables, lead

concentration and length of exposure. On the dependent variables, pair-wise mean comparisons were conducted, using Student *t*-test. A replicated analysis using multiple regressions was conducted to generate regression model.

Results and Discussion

The results show that there was significant ($P < 0.05$) difference in homogenate protein lead content between control and experimental groups. This was also true of microsomal protein content. Further hepatic homogenate lead levels showed a progressive increase with increasing lead concentration Table 1 contains the results of the analysis of multi-variance (MANOVA) As shown from MANOVA table the main effects of length of time and concentration were highly significant ($P < 0.001$), which means that the difference between the overall mean and the means of all potential group means is different Thus lead accumulated in the liver and microsome was significantly affected by the length of time of exposure to lead and level of concentration for all parameters Figure 1 illustrates that the bio-accumulated lead in liver, erythrocytes and plasmas were correlated with time of exposure Data were fitted using multiple curvilinear regression as such the best fit model was generated. All regression models are embedded in their corresponding figures The best fit model was selected based on minimum least square criterion, and minimum standard error of estimation The results of multiple regression indicate that the explained variance of hepatic, erythrocytes, and plasma lead contents accounted for by length of exposure is significant ($P < 0.5$).

Cytochrome system changes : Table 2 shows that with progressive increase in lead treatment there was an associated progressive rise in lead contents in liver, Erythrocyte, and plasma, for all periods of exposure. Pair wise mean comparisons revealed significant difference in the lead-treated animals of groups exposed to 7 and 14, 28, and 45 days that were exposed to 8, 15, and 20 $\mu\text{gm/l}$, respectively with respect to their corresponding controls. It was also noted that the activity of hepatic ALA

Table - 1 : Multifactor analysis of variance (MANOVA) that clear the effect of time, exposure levels and time/ lead (Pb) interaction on different studied Parameters. The degree of freedom (df) of error=80, df of total= 100 and df of corrected total= 99.

Variable	Sum of squares	df	Mean square	F-ratio	P-value
Time effect on :					
Hepatic Pb content	560.502	4	140.126	1564	P < 0.001
Plasma Pb level	21.388	4	5.347	307	P < 0.001
RBCs Pb content	3.742	4	0.936	314	P < 0.001
Hepatic Fe content	6436.086	4	1609.022	6	P < 0.001
Plasma Fe levels	49887.140	4	12471.785	354	P < 0.001
RBCs enumeration	7.526	4	818.374	818	P < 0.001
WBCs enumeration	153.480	4	953.410	953	P < 0.001
Hb concentration	218.898	4	638.555	639	P < 0.001
Blood glucose levels	73568.260	4	18392.065	1413	P < 0.001
Hepatic glycogen content	1.685	4	545.035	545	P < 0.001
AST activity	33261.987	4	8315.479	305	P < 0.001
ALT activity	15170.976	4	3792.744	285	P < 0.001
δ-ALAD activity	25131.160	4	6282.790	358	P < 0.001
Lead effect on :					
Hepatic Pb content	812.118	3	270.706	3022	P < 0.001
Plasma Pb level	70.802	3	23.601	1353	P < 0.001
RBCs Pb content	6.322	3	2.107	708	P < 0.001
Hepatic Fe content	90199.704	3	30066.568	115	P < 0.001
Plasma Fe levels	68353.200	3	22784.400	648	P < 0.001
RBCs enumeration	1.269	3	0.423	184	P < 0.001
WBCs enumeration	137.282	3	45.761	1137	P < 0.001
Hb concentration	87.303	3	29.101	340	P < 0.001
Blood glucose levels	26435.150	3	8811.717	677	P < 0.001
Hepatic glycogen content	2.104	3	0.701	908	P < 0.001
AST activity	160103.257	3	53367.752	1958	P < 0.001
ALT activity	109013.508	3	36337.836	2734	P < 0.001
δ-ALAD activity	52544.430	3	17514.810	997	P < 0.001
Time/lead interaction on :					
Hepatic Pb content	46.607	12	3.884	43	P < 0.001
Plasma Pb level	18.443	12	1.537	88	P < 0.001
RBCs Pb content	2.715	12	0.226	76	P < 0.001
Hepatic Fe content	10935.363	12	911.280	911	P < 0.001
Plasma Fe levels	18135.900	12	1511.325	1511	P < 0.001
RBCs enumeration	2.554	12	0.213	93	P < 0.001
WBCs enumeration	41.540	12	3.462	86	P < 0.001
Hb concentration	63.829	12	5.319	62	P < 0.001
Blood glucose levels	39053.900	12	3254.492	250	P < 0.001
Hepatic glycogen content	0.987	12	0.08224	106	P < 0.001
AST activity	12157.482	12	1013.123	37	P < 0.001
ALT activity	5908.193	12	492.349	37	P < 0.001
δ-ALAD activity	12324.520	12	1027.043	59	P < 0.001

***, P < 0.001, very high significant difference in comparison to the corresponding control.

synthase in the lead-treated fish was increased significantly (P < 0.05) which was associated with

decreased microsomal P-450 content (Fig. 2). The continuous accumulation of lead results in a

progressive significant lowering of cytochrome P-450 content. This depression was significant in the lead-treated fish of groups 2, 3 and group 4 with respect to their appropriate controls (Table 2).

According to MANOVA results, the exposure time to lead caused a significant increase in ALA synthase activity ($F_{4,20} = 76.8$, $P < 0.001$) and a decrease in cytochrome P-450 content ($F_{4,20} =$

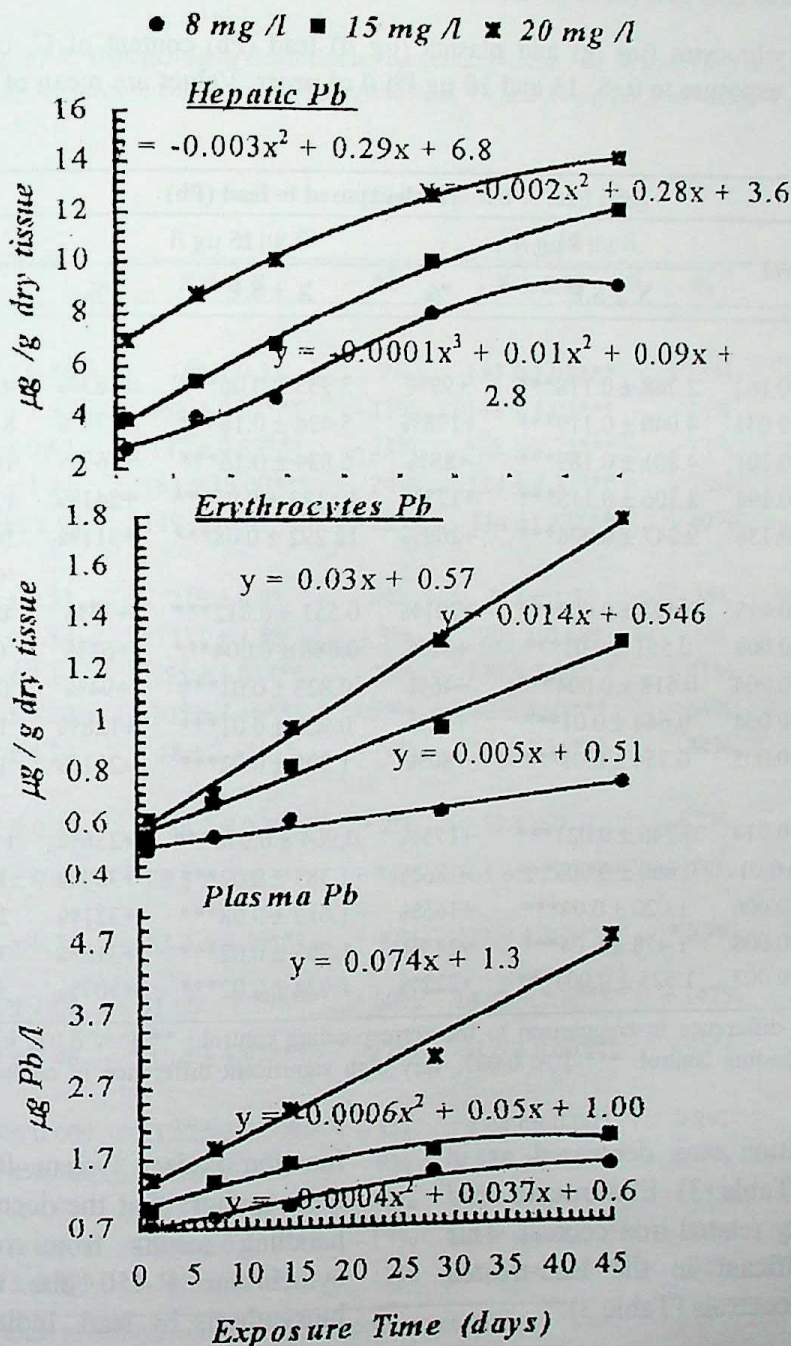


Fig. 1 : Changes in hepatic, erythrocyte ($\mu\text{g Pb/g}$) and serum ($\mu\text{g Pb/l}$) lead content of *C. carpio* during 45 days of continuous exposure to different levels of lead in water of aquaria. Each point represents mean of five fish.

45.6, $P < 0.001$). The decrease of ALA synthase activity and cytochrome P-450 were correlated with the bio-accumulated lead in the liver (Fig. 2,

3). Regression analysis showed inverse relationship between ALA synthase and cytochrome P-450 reveals a highly significant.

Although, like the cytochrome P-450 content, cytochrome b5 content was decreased with increasing exposure time to lead, the effect was not so marked and only attained statistical significant in the lead-treated fishes (Table 2).

Blood chemistry changes : The hepatic homogenate accumulated lead content was negatively correlated with ALAD activity, hepatic glycogen, and blood glucose, (Fig. 3).

Table - 2 : Hepatic, erythrocytes ($\mu\text{g/g}$) and plasma ($\mu\text{g/l}$) lead (Pb) content of *C. carpio*, during 45 days of continuous exposure to 0, 8, 15 and 20 $\mu\text{g Pb/l}$ of water. Values are mean of five fish \pm standard error ($X \pm \text{S.E.}$).

Time	Fish exposed to lead (Pb)						
	Control	8 µg /l		15 µg /l		20 µg /l	
		X ± S.E.	%	X ± S.E.	%	X ± S.E.	%
Liver:							
1 day	1.389 ± 0.162	2.768 ± 0.118***	+99%	3.933 ± 0.06***	+183%	6.999 ± 0.05***	+404%
7 days	1.449 ± 0.035	4.040 ± 0.119***	+178%	5.424 ± 0.18***	+274%	8.782 ± 0.157***	+489%
14 days	2.561 ± 0.201	4.801 ± 0.189***	+88%	6.834 ± 0.16***	+167%	10.113 ± 0.06***	+295%
28 days	2.968 ± 0.198	8.106 ± 0.115***	+173%	10.123 ± 0.07***	+241%	12.781 ± 0.12***	+331%
45 days	2.991 ± 0.138	9.247 ± 0.076***	+209%	12.292 ± 0.08***	+311%	14.350 ± 0.83***	+380%
Erythrocyte:							
1 day	0.416 ± 0.019	0.497 ± 0.002***	+201%	0.551 ± 0.012***	+33%	0.624 ± 0.01***	+50%
7 days	0.421 ± 0.009	0.551 ± 0.01***	+31%	0.688 ± 0.004***	+63%	0.719 ± 0.01***	+71%
14 days	0.424 ± 0.004	0.618 ± 0.004***	+46%	0.823 ± 0.01***	+94%	0.974 ± 0.02***	+130%
28 days	0.425 ± 0.004	0.644 ± 0.01***	+52%	0.968 ± 0.01***	+128%	1.310 ± 0.05***	+208%
45 days	0.420 ± 0.005	0.754 ± 0.01***	+80%	1.305 ± 0.02***	+211%	1.798 ± 0.08***	+328%
Plasma:							
1 day	0.271 ± 0.014	0.746 ± 0.021***	+175%	0.964 ± 0.018***	+256%	1.407 ± 0.091***	+419%
7 days	0.301 ± 0.01	0.860 ± 0.009***	+186%	1.383 ± 0.02***	+360%	1.852 ± 0.06***	+515%
14 days	0.385 ± 0.006	1.020 ± 0.03***	+165%	1.619 ± 0.08***	+321%	2.388 ± 0.05***	+520%
28 days	0.424 ± 0.008	1.478 ± 0.04***	+249%	1.765 ± 0.02***	+316%	3.086 ± 0.06***	+628%
45 days	0.473 ± 0.003	1.525 ± 0.001***	+222%	1.924 ± 0.02***	+307%	4.757 ± 0.20***	+906%

$P < 0.05$, high significant difference in comparison to the corresponding control; ** $P < 0.01$, high significant difference in comparison to the corresponding control. *** $P < 0.001$, very high significant difference in comparison to the corresponding control.

Iron concentration was decreased as in hepatic and plasma (Table 3) Erythrocyte lead contents, was negatively related iron content. This was statistically significant in the lead-treated groups as compared to controls (Table 3).

Results of previous studies on animal (Alvares *et al.*, 1972; Scoppa *et al.*, 1973; Ratle, 1999) and on human (Alvares *et al.*, 1975; Meredith *et al.*, 1977) have demonstrated that lead inhibits the elimination of drugs metabolized by the cytochrome P-450 dependent microsomal mixed

function oxidase system. It has been suggested by these authors that the decreased capacity for drug handling results from reduced availability of cytochrome P-450 due to inhibition of haem biosynthesis by lead. Indirect evidence of such a mechanism has been obtained by measuring inhibition of the activity of the haem biosynthetic enzyme ALA dehydratase (Alvares *et al.*, 1972; Scoppa *et al.*, 1973; Meredith *et al.*, 1977). It has also been suggested that the lead exerts its action directly on the hepatic mixed function oxidase system (Alvares *et al.*, 1972).

Results from the present study provide unequivocal evidence that lead administration in fish results in decreased cytochrome P-450 content and inhibition of demethylase and hydroxylase activities and that this is associated with a

depression by lead of haem biosynthesis. Increasing lead bioaccumulation resulted in a progressive decrease in cytochrome P-450 content and a progressive decrease in the activities of aniline hydroxylase and aminopyrine demethylase.

Table - 3 : Hepatic iron ($\mu\text{g/g}$) & glycogen (g/g fresh tissue) contents, plasma ($\mu\text{g/l}$) iron (Fe) and blood glucose concentration (mg/dl) and during 45 days of continuous exposure to 0, 8, 15 and 20 $\mu\text{g Pb/l}$ of water. Values are mean of five fish \pm standard error ($\text{X} \pm \text{S.E.}$).

Time	Fish exposed to lead (Pb)						
	Control	8 µg /l		15 µg /l		20 µg /l	
		X ± S.E.	%	X ± S.E.	%	X ± S.E.	%
Hepatic iron							
1 day	176 ± 0.46	164 ± 0.53	- 7%	153 ± 1.93***	- 13%	133 ± 1.77***	- 24%
7 days	182 ± 1.83	158 ± 0.93***	- 13%	138 ± 1.02***	- 24%	119 ± 1.07***	- 35%
14 days	191 ± 0.93	147 ± 0.37***	- 23%	128 ± 1.72***	- 33%	108 ± 1.21***	- 44%
28 days	201 ± 1.21	153 ± 13.00***	- 24%	124 ± 4.37***	- 38%	101 ± 5.20***	- 50%
45 days	191 ± 22.95	149 ± 16.61***	- 22%	114 ± 1.47***	- 40%	73 ± 3.00***	- 62%
Plasma (Iron)							
1 day	219 ± 0.51	219 ± 0.93	0%	217 ± 1.36	- 1%	215 ± 1.64	- 2%
7 days	218 ± 1.84	211 ± 1.58	- 3%	201 ± 0.68*	- 8%	178 ± 2.07***	- 18%
14 days	219 ± 3.14	155 ± 3.03***	- 29%	130 ± 2.84***	- 41%	107 ± 3.15***	- 51%
28 days	232 ± 3.22	105 ± 3.16***	- 55%	83 ± 4.97***	- 64%	63 ± 3.18***	- 73%
45 days	228 ± 4.24	48 ± 2.55***	- 79%	37 ± 1.54***	- 84%	23 ± 2.10***	- 90%
Blood glucose							
1 day	58.2 ± 0.37	59.2 ± 0.37	+ 2%	59.4 ± 0.51	+ 2%	62.6 ± 1.89*	+8%
7 days	59.0 ± 0.45	78.8 ± 2.48***	+ 34%	105.8 ± 2.25***	+ 79%	120.0 ± 0.08***	+103%
14 days	59.4 ± 0.25	83.2 ± 0.86***	+ 40%	113.0 ± 2.00***	+ 89%	131.0 ± 1.00***	+121%
28 days	57.2 ± 1.02	91.60 ± 1.36***	+ 60%	141.2 ± 2.87***	+ 147%	165.0 ± 1.75***	+189%
45 days	58.0 ± 3.48	42.4 ± 1.97***	- 27%	31.6 ± 0.60***	- 46%	21.60 ± 0.51***	- 63%
Hepatic glycogen							
1 day	1.643 ± 0.009	1.625 ± 0.008	- 1%	1.619 ± 0.005	- 2%	1.617 ± 0.007	- 2%
7 days	1.664 ± 0.020	1.526 ± 0.005*	- 8%	1.514 ± 0.007**	- 9%	1.475 ± 0.008**	- 11%
14 days	1.643 ± 0.010	1.473 ± 0.004**	- 10%	1.393 ± 0.004***	- 15%	1.245 ± 0.020***	- 24%
28 days	1.683 ± 0.009	1.406 ± 0.009***	- 17%	1.238 ± 0.020***	- 26%	1.076 ± 0.030***	- 36%
45 days	1.669 ± 0.009	1.348 ± 0.009***	- 19%	1.128 ± 0.020***	- 32%	0.912 ± 0.010***	- 45%

* $P < 0.05$, Significant difference in comparison to the corresponding control.

** $P < 0.01$, High significant difference in comparison to the corresponding control.

*** $P < 0.001$, very high significant difference in comparison to the corresponding control.

As has been noted (Maxwell and Meyer, 1976), associated with this impairment of the mixed function oxidase system there was an increase in the activity of the enzyme ALA synthase. These mitochondrial enzyme catalyses the initial step of

the haem biosynthetic pathway and is the decisive controlling factor for overall rate of haem synthesis in the liver (Marver and Schmid, 1972). The activity of this enzyme to be regulated by the end of product of the pathway, haem, and for this

reason a regulatory 'pool' of hepatic haem has been postulated (De Matteis, 1971; Tschudy and Bonkowsky, 1972; Meyer and Schmid, 1973; Watson, 1975). The precise mechanism of this regulation remains controversial (Scholnick *et al.*, 1969; Sassa and Granick, 1970; Hayashi *et al.*, 1972), although evidence for its existence has come from various studies. When haem was

administered to rats (Marver *et al.*, 1968; Watson, 1975), or added to the incubation medium of cultured chick embryo liver cells (Granick, 1966; Sassa and Granick, 1970; Strand *et al.*, 1972). It blocks the response of ALA synthase to inducing chemicals such as phenobarbitone or allyl isopropylacetamide.

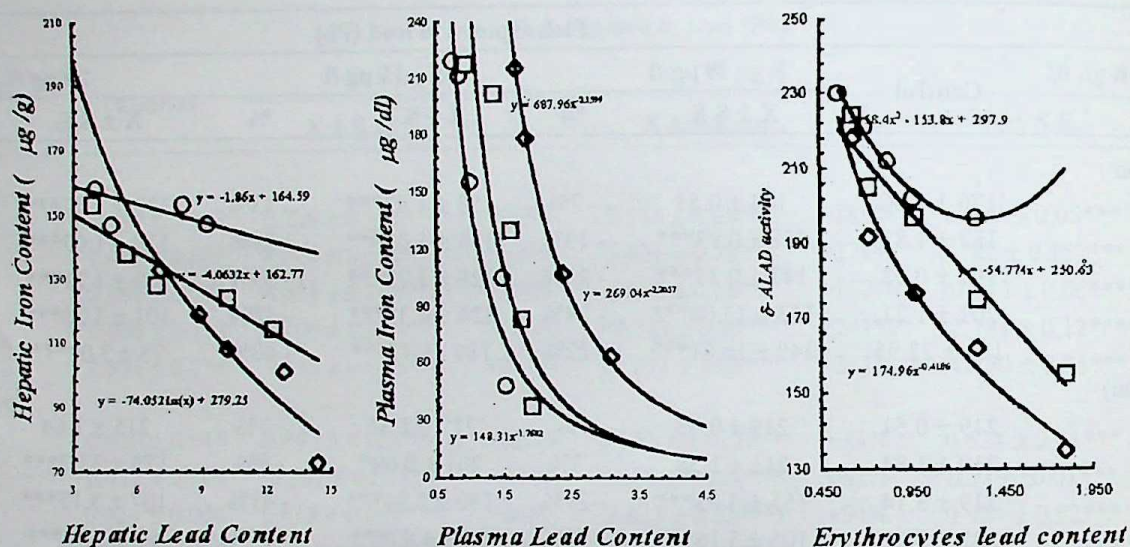


Fig. 2 : Relationship between hepatic lead and iron content; plasma iron and lead content; erythrocytes lead content and erythrocytes δ-ALAD activity of *C. carpio* after 1,7,28,45 days of continuous exposure to 8,15 and 20 µg Pb/l of water in aquarium. Each point represents mean of five fish.

The inverse relationship between ALA synthase and cytochrome P-450 content noted in the present study provides further evidences of the control mechanism whereby a decrease in the regulatory 'pool' of haem, as assessed by the microsomal content of the haemoprotein cytochrome P-450, results in an increase in the activity of ALA synthase activity. The decrease in the microsomal content of cytochrome P-450 associated with lead pretreatment can be attributed to the decrease in the activities of the haem biosynthetic enzymes ALA dehydratase, COPRO oxidase and ferrochelatase reducing the available free haem for cytochrome P-450 synthesis. It is interesting to note that these results obtained from the livers in fish treated with lead follow a similar pattern to that observed in the peripheral blood of a group of lead workers (Campbell *et al.*, 1977).

The activity of the microsomal haem degradative enzymes, haem oxygenase was found to be increased as the pretreatment of the animals with lead increased. This is in agreement with the findings of Maines and Kappas (1976a) but conflicts with the model suggested by Bissell and Hammaker (1976). Their model considered that ALA synthase and haem oxygenase are inter-related with respect to their regulation by haem. They further predicted that these enzymes activities vary reciprocally, this theory having experimental support in the studies of Schacter (1975). From this theory, one would expect depletion of the regulatory haem, which would activate ALA Synthase but depress haem oxygenase. In the present study the experimental evidence does not support this theory, for as lead pretreatment of the rats inhibits hepatic haem

Effect of lead bioaccumulation on fish.

synthesis and thereby depletes the regulatory haem, there is not only associated increase in the activity of ALA synthase but also a rise in the activity of haem oxygenase.

The results of the present study are consistent, however, with the theory postulated by Maines and Kappas (1976b) that the mode of action of metals in inducing haem oxygenase is based on a suppressor component in the regulatory

mechanism of haem oxygenase. They suggested that this repressor has an -SH active constituent, the oxidation-reduction capacity of which is necessary for controlling haem oxygenase production. If the oxidation reduction cycle of the -SH groups of this components is blocked, as would occur after treatment with lead which has a high affinity for -SH groups, the regulatory function of this cellular constituents on haem

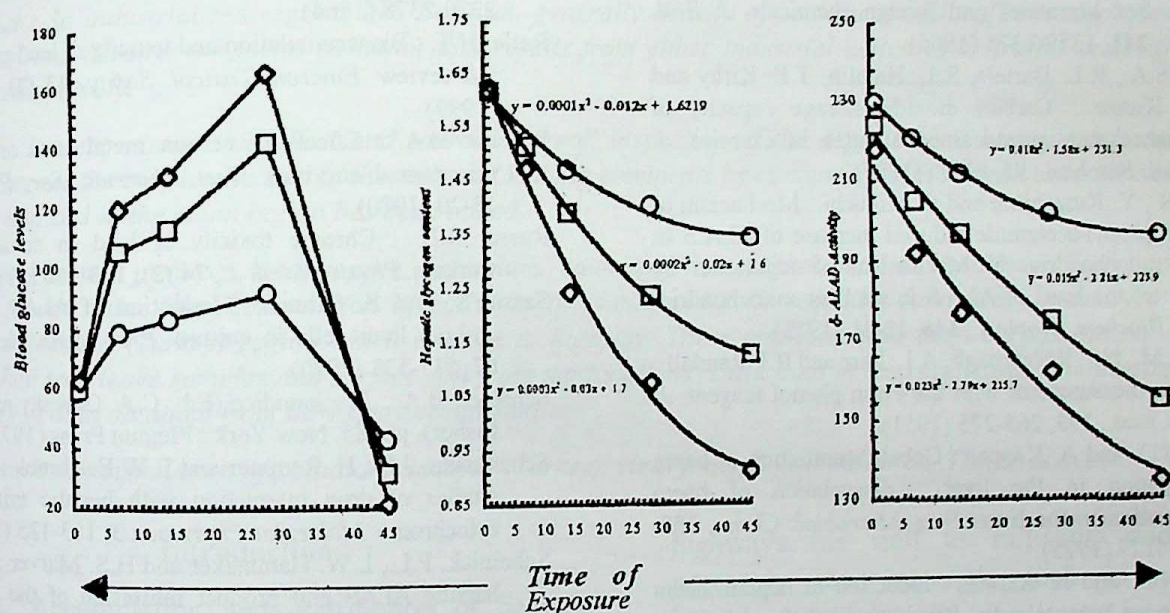


Fig. 3 : Changes of blood glucose levels, hepatic glycogen content and erythrocytes d-ALAD activity of *C. carpio* after 1,7,28,45 days of continuous exposure to 8,15 and 20 mg Pb.

oxygenase would be lost. The system would then function without repressive regulation, leading to an exaggerated synthesis of the enzyme.

The present work provides some explanation with regard to the relative importance of inhibition of haem synthesis and of accelerated haem degradation in the reduction of microsomal cytochrome P-450 content following lead administration.

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Research of usability of tree leaves and soil in determining the contribution of industry and traffic to air pollution in Bozüyük (Turkey) region.

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Abstract : *In industrialized regions like Bozüyük, generally density of settlement and traffic is also observed. As a result of this density, the metal pollution that results from either industrial activities or traffic shall affect the air quality negatively.*

In determining this effect and sources thereof, inspection of the depositing of heavy metals, which cause pollution, on the tree leaves and in the soil, and making comments by comparing with the values in the same kinds of plants and soil in the clean region has been aimed.

For this purpose, zinc, copper, chromium, cadmium, iron, nickel, lead analysis have been carried out in order to determine the accumulation of pollution in plants and soils resulting from heavy industry and vehicles around Bozüyük (Turkey) region which is close to highway. These analyses have been carried out on washed and unwashed tree leave samples and surface soil from ten locations. Data used in the results were the average values of a series of data obtained from the experimental studies.

Key words : *Accumulation of pollution, Bozüyük (Turkey), Heavy metal, Soil pollution, Traffic activities, Tree species.*

Introduction

Accumulation and distribution of heavy metals from the environment are well-documented features of botanical materials such as lichens, moss and part of the higher plants, have been used to monitor atmospheric depositions of a large number of metals. Work in the past few decades suggested marked accumulation of heavy metals in tree leaves and soils close to industrial areas and heavily traffic highways (Galun, 1988; Market, 1993; Aksoy *et al.*, 2000). In this work heavy metal contamination in Bozüyük (Turkey) was investigated.

Bozüyük, which lies between the southeastern part of them Marmara region and the Northwest Middle-Anatolia, was every time a residential district since the Old Phrygians (B.C.) and has been industrialized rapidly during the last 15 years. A great movement of industrialization began in the town, as; it lies at the junction of the Istanbul-Eskişehir-Ankara and Ankara-Bursa

highways (as well as railways) and has the necessary raw materials such as manganese, lignite, chromium, and clay layers in the region. The industrialization of the town began with the ceramic production and continued with the productions of paper, cable, biscuit, sponge, plaster, radiator, armature, thread, carpet, tile and electric bulb (approximately 35 factory).

Materials and Methods

Ecological properties of the research area : The altitude of Bozüyük is 743 m. The area is surrounded by Yirce Mountain (1790 m) in the west, Kızıltepe Hill (900 m) in the north, Osmaniye Hill (1210 m) and Kızılcaviran Hill (1250 m) in the north and northwest, Kala Mountain (1906 m) in the southwest. The mean temperature and annual precipitation are 12°C and 430 mm, respectively. Prevailing winds are north and northwest. Annual relative humidity is 72.5 % (Anonymous, 2000-2001). Wheat, beet, sunflower,

corn and potato are widely cultivated (29.2 % of the surface area) in this region. Main tree species, especially mountainous areas *Pinus nigra* subsp.

pallasiana (Lamb.) Holmboe., *Pinus sylvestris* L., *Quercus cerris* L., *Quercus pubescens* L., *Quercus infectoria* L., *Abies nordmanniana*

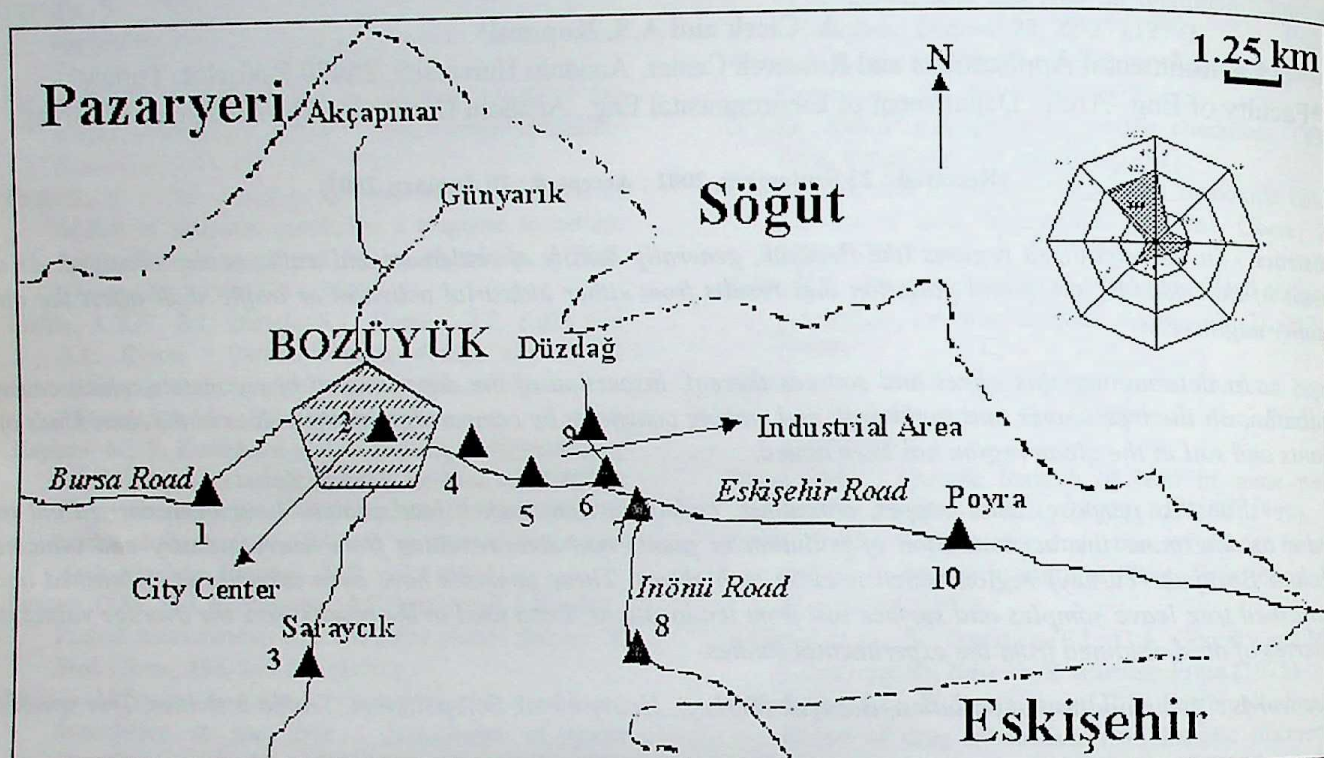


Fig. 1 : Sampling plots in the research area.

Table - 1 : Locations of the collected tree leave and soil samples.

Loc. No	Features of Locations and Tree Species
1	Bozüyük-Bursa highway 1 st km, 717m, <i>Pinus nigra</i>
2	Bozüyük, residential area, 754m, <i>Salix babylonica</i>
3	Saraycık Village, 813m, <i>Salix babylonica</i>
4	Bozüyük-Eskişehir highway 2 nd km, 769m, <i>Pinus nigra</i>
5	Bozüyük-Eskişehir highway 3 rd km, 766m, <i>Populus tremula</i>
6	Bozüyük-Eskişehir highway, 4 th km, 755m, <i>Salix babylonica</i>
7	Bozüyük-Eskişehir highway, 5 th km, 755m, <i>Populus tremula</i>
8	Bozüyük-Inönü highway, 2 nd km, 772m, <i>Quercus infectoria</i>
9	Düzdağ Village road, 1 st km, 760 m, <i>Quercus infectoria</i>
10	Poyra Village, in the vicinity of the highway, 939m, <i>Pinus nigra</i>

subsp. *bornmülleriana* (Mattf.), *Carpinus betulus* L., *Populus tremula* L., *Fraxinus* sp. L., *Acer* sp. L. are distributed (Anonymous, 1998).

Tree leave and soil samples used in this investigation were collected from 10 locations (Fig.

1 and Table 1) between October-November 2001 in Bozüyük (Bilecik/Turkey) Region.

The unwashed and washed leave samples were dried at 105°C for 24h. Surface soils (0-20cm) were dried at 105°C for 3h. After all

samples digestion were carried out by using a mixture of concentrated HNO_3 and HClO_4 (CEM Star 2 Model microwave digestion unit); then, by dissolving the precipitate in HCl 0.5n. The total content of heavy metals was determined by AAS (Varian Spectra A 250 Plus Model) method (ASTM, 1985; APHA, 1992).

Results and Discussion

The concentrations of heavy metals (zinc, copper, iron, lead, chromium, nickel, cadmium) in unwashed and washed leaves are given in Figs. 2-8, respectively.

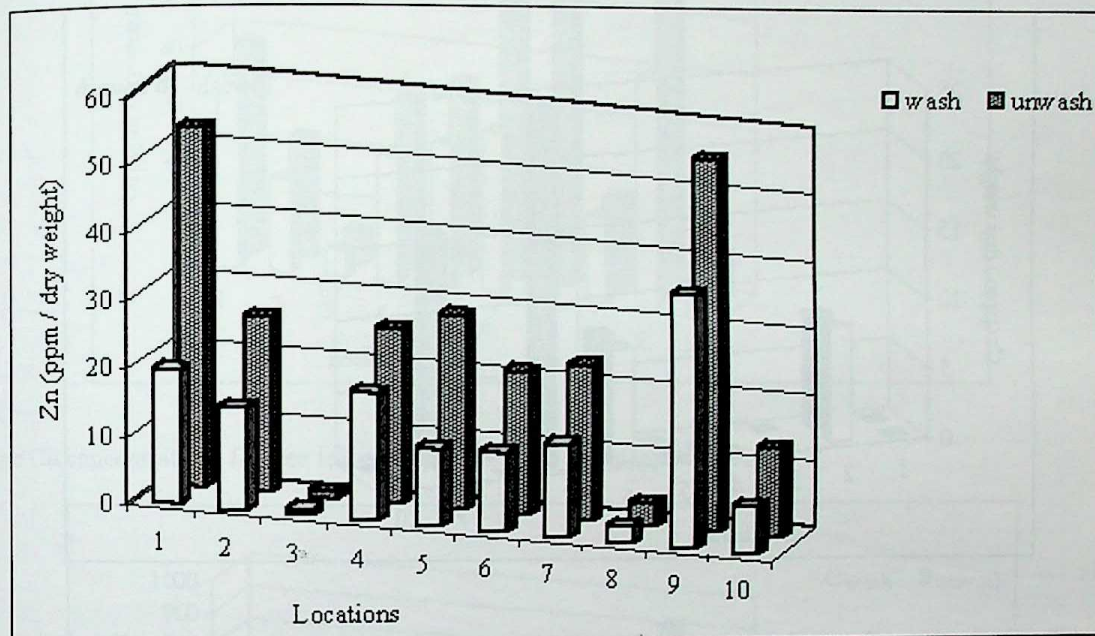


Fig. 2 : Average Zn concentrations for tree leaf samples from the investigated area.

As it is also seen in Fig. 1, location 3 is far from the Bozüyük settlement area. It has been chosen as the region where industry and traffic doesn't exist. The zinc concentration in location 3 is lower according to the other locations; however, it has the same values in the washed and unwashed samples. The locations 2, 4, 5, 6, 7 and 10 are regions where the traffic is dense and the zinc concentrations are high. The values' in the unwashed samples being high indicates that the zinc resulting from traffic is carried onto the plants through the air. The results of the soil analyses in Table 2 are also relevant to this. The location 8 is the place where the traffic is relatively less and the zinc level is also lower. Moreover, this location has a position where the effect of the automotive industry can be partially felt. The location 1 is on the highway of Bursa, which is one of the biggest

provinces of Turkey and which is one of the most important harbor centers, and it has very dense traffic. As it is seen in Fig. 2, the highest zinc concentrations have been found in the location 9. This region is the place where ceramic, cable and metal processing industries are dense and together with the contribution of the other industries, it has been determined that there is significant zinc pollution in this location.

When the region was inspected from the aspect of chromium, it is seen that chromium concentrations are high in the location 9 and location 7 where industry is dense. In these locations, the unwashed sample values are higher according to the washed ones. Wire drawing and chromium plating facilities are located between the locations 6 and 7. Since the location 6 is in the opposite of the prevailing wind direction, it is not

affected from chromium pollution as much as the location 7. The chromium concentrations' in the washed and unwashed samples in location 6 being the same supports the fact that chromium accumulation to location 7 is high since it is on the prevailing wind direction. The location 2 is in Bozüyük urban center and it can be stated that the

determined chromium results from small-scaled workshops and daily activities. Other locations do not contain chromium like the reference location 3.

When the region is inspected from the aspect of cadmium pollution, the effect of dense ceramic factories is observed in the location 9. A

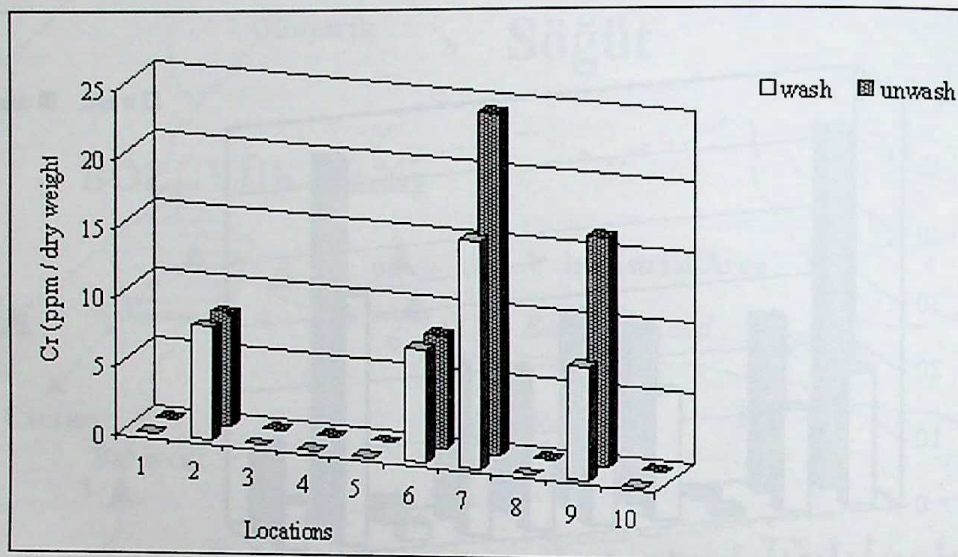


Fig. 3 : Average Cr concentrations for tree leave samples from the investigated area.

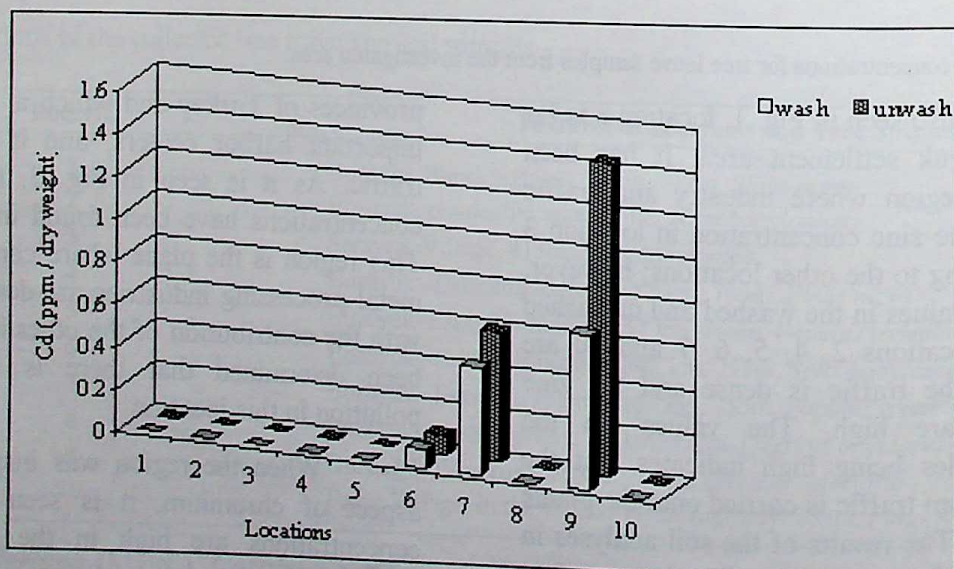


Fig. 4 : Average Cd concentrations for tree leave samples from the investigated area.

considerable part of ceramic production of Turkey (Turkey is one of the first five countries in ceramic production throughout the world) is realized in this location. Pollution has been determined also in

location 7 that is near the region and where small-scaled ceramic facilities are located. The pollution level is relevant to the ceramic factory density. Similar situation is observed in location 6 and the

Usability of tree leaves and soil in determining the air pollution.

rarely seen cadmium levels are in approximately the same value for washed and unwashed samples in this region, which is in the opposite of the prevailing wind direction.

General distribution of copper concentrations is seen in Fig. 5. In this distribution, which indicates parallelism with other metal pollutants, the place where copper is most dense is

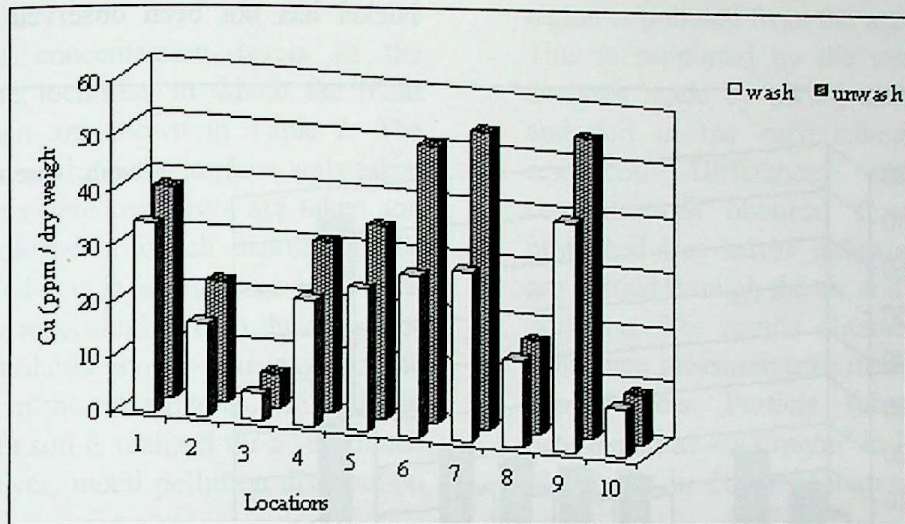


Fig. 5 : Average Cu concentrations for tree leave samples from the investigated area.

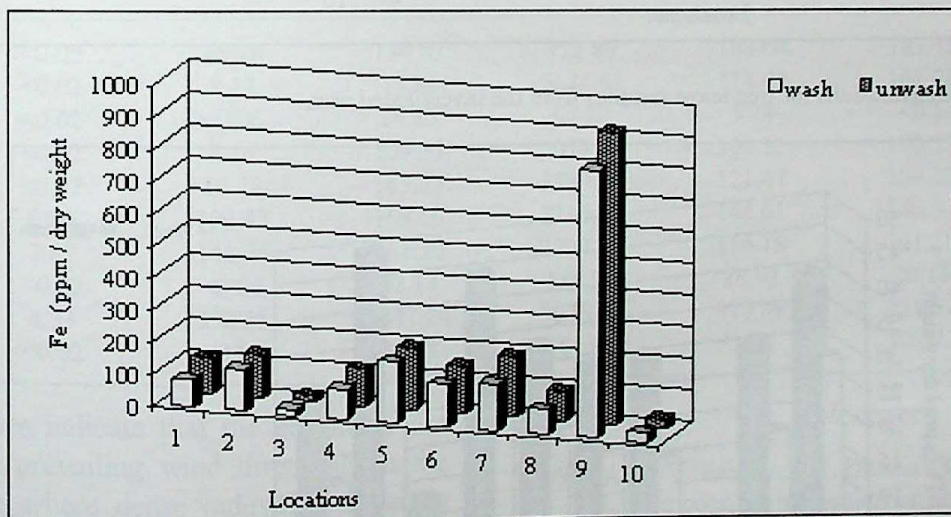


Fig. 6 : Average Fe concentrations for tree leave samples from the investigated area.

the locations where the industry region no. 9 and the copper cable factory no. 6 are located. Locations 5 and 7 that are chosen near the copper factory are places where copper pollution is relatively high. The urban area (location 2) is exposed to copper pollution both due to the traffic inside the city and the city activities. The localities other than those in the reference location 3 and

especially the dense traffic area (location 1) include copper resulting from traffic.

It is seen that in general the region has values between 15-200 ppm from the aspect of iron, however, this value reaches to very high Figures like 900 ppm in the dense industry region (location 9). The data obtained from locations 5 and 6, where wire drawing workshops are located,

and location 2, where small-scaled workshops are located inside the city, are parallel to the activities in these regions. The relatively low iron concentrations in the other locations and in the reference region (location 3) are indicated in Fig. 6.

In Fig. 7, it is seen that nickel pollution reaches to very high levels in the industry region (location 9). Location 2 that is the urban region and locations 4, 5, 6, 7 and 8 that are near the industry region are exposed to nickel pollution. Nickel has not been observed in the location 3

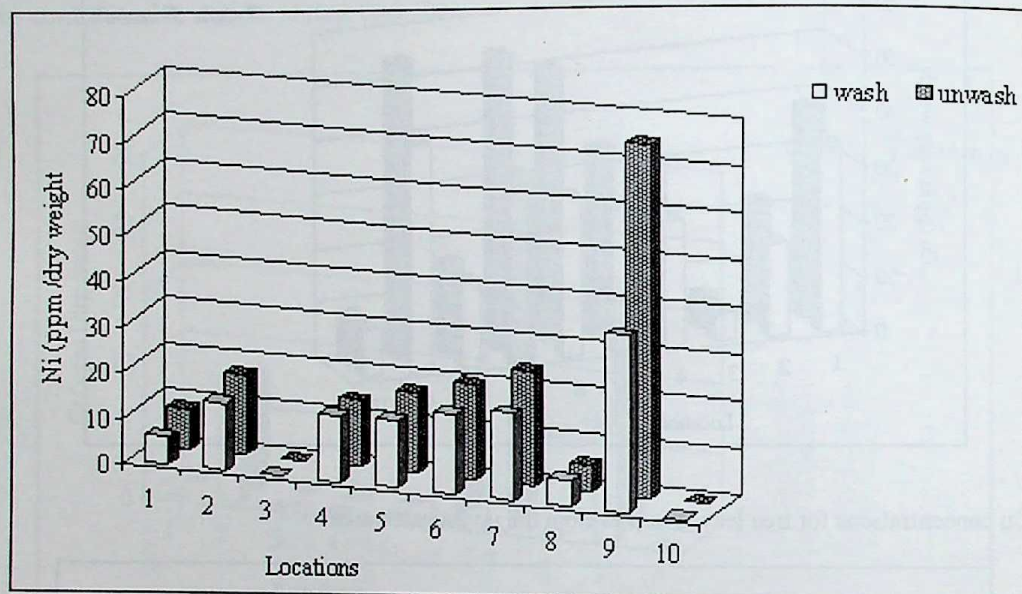


Fig. 7 : Average Ni concentrations for tree leave samples from the investigated area.

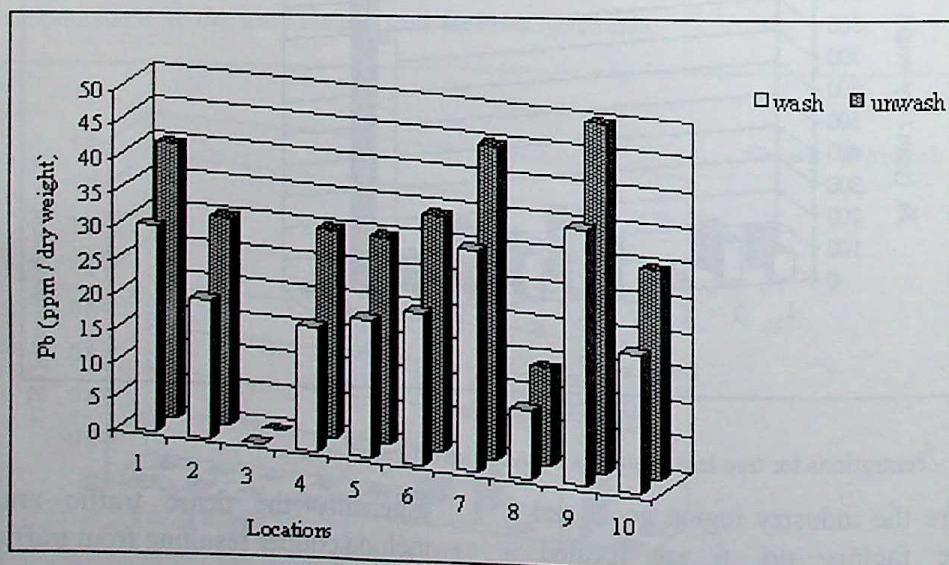


Fig. 8 : Average Pb concentrations for tree leave samples from the investigated area.

chosen as clean area and location 10 that is far from industry.

As it is clearly seen from Fig. 8, lead pollution is distributed related to the traffic. Lead

pollution increases with the effect coming from industry in the industry regions 7 and 9, traffic density is seen in region 1 with dense traffic and in locations 4, 5 and 6 that are near the industry

region. The lead pollution seen in the urban centers is related to the dense traffic activities in the highway passing through the center of the city. Lead pollution is not observed in location 3 as the other metals.

All metal concentration levels in the surface soil at the localities in which the plant samples were taken are shown in Table 2. The results of analyses made in the surface soils taken from the locations where tree laves are taken and results of the analyses in which deposit on the leaves are inspected are in accordance with each other. Values very much higher than the values of heavy metal accumulated on one-year leaves of the trees are observed in the soil samples. This is since the accumulation in soil is realized for a very much longer time. However, metal pollution distribution

trends in plants and soil according to the regions are in accordance with each other.

The results obtained from the study made in Bozüyük (Turkey) region indicate that the region is polluted from the aspect of heavy metals. This is supported by the relevance between the analyses made by taking samples from the trees and soil in the surroundings of the polluting resources. Differences between the metal concentrations obtained from the washed and unwashed tree leaves indicate that the pollutants are carried through the air and that there is particle pollution. The results obtained from the particle substance measurements made in the region also support this. Particle substance amount was determined as $40\text{--}50\mu\text{g}/\text{m}^3$ in Bozüyük in October-November in 2001. Moreover, the data obtained

Table - 2 : Heavy metal concentrations in soil samples (ppm/dry weight).

Loc. no	Cd	Cr	Cu	Fe	Ni	Pb	Zn
1	<0.02	16.28	134.85	722.89	133.04	181.18	187.70
2	<0.02	8.14	75.84	5684.61	114.03	161.28	37.81
3	<0.02	<0.06	16.85	69.20	5.78	<0.1	11.47
4	<0.02	8.14	109.55	2053.77	104.12	100.73	39.67
5	<0.02	16.28	143.27	3380.63	121.47	100.73	38.74
6	2.89	130.42	168.56	3215.77	144.61	140.97	40.60
7	2.89	146.74	261.30	2297.84	156.18	161.28	55.99
8	<0.02	<0.06	42.13	584.96	28.92	20.16	8.25
9	4.33	392.15	2841.24	5076.92	810.08	221.37	449.98
10	<0.02	<0.06	25.28	584.96	5.78	100.73	6.88

from the analyses indicate that the pollutants are effective in the prevailing wind direction. In the Bozüyük region where dense industrial activities are realized that are mingled with the urban area and where the traffic density in 18000-20000 vehicle/day, as a result of the comparisons made with the reference samples taken from clean regions, the existence of the metal pollution that we had estimated previously has been brought to light.

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Effect of kinetin on leaf protein content and its profile in mung bean under salt stress.

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Abstract : Application of NaCl resulted in about 67% reduction in amino acid content and 24% reduction in buffer soluble protein content in mung bean [*Vigna radiata* (L.) Wilczek.] leaf as compared with the control. Gel electrophoretic profile of buffer soluble protein content in leaf of mung bean showed an extra band in between 29 kD and 45 kD in stress protein profile as compared with control. It was noted that the foliar spray of kinetin (6-furfuryl aminopurine) used in the present study was able to overcome upto certain extent the adverse effects of stress caused by NaCl.

Key words : Kinetin, Amino acid, Protein, Mung bean, Salt stress.

Introduction

Salinity of soil leads to metabolic alterations and graded reduction in plant growth. This could be due to decrease in osmotic potential of the medium, disturbance in the mineral nutrition of the plant and direct toxic effects of saline conditions on plant growth and metabolism through exchange of cations and anions in plant cells (Munns and Termaat, 1986). In Pigeon pea, soluble protein content decreases at salinity levels of 7.25 dS/m and 9.00 dS/m (Gill and Sharma, 1993). Salts in the medium, suppress the cowpea callus growth and reduction in soluble amino acids content (Agarwal and Gupta, 1995). In *Sporobolus spicatus* and *Cyperus laevigatus*, salinity affects free amino acids and crude protein concentration (Onkware, 1997). Ericson and Alfinito (1984) reported that when the protein pattern of NaCl adapted cultured tobacco cells was compared to that of unadapted cells, 32 kD and 20 kD protein bands were much more abundant and 26 kD protein band was unique to the adapted cells. In high-proline producing variant of rice, NaCl stress increases total free amino acid content and 53.3 kD protein content when compared with the original type (Dao-Yao and Shu-Wen, 1995). In mung bean, salt stress reduces amino acid and

protein concentration. GA₃ can overcome to variable extents the adverse effects of stress imposed by NaCl solution (Chakrabarti and Mukherji, 2002).

The present study investigates the effect of salt stress on amino acid content and on buffer soluble protein content and its Gel electrophoretic profile in mung bean leaf and also the efficiency of kinetin (6-furfuryl aminopurine) in restoring the metabolic alterations resulting from salt stress.

Materials and Methods

A pot study on mung bean (*Vigna radiata* cv. B105) was conducted in sandy-loam soil without adding fertilizer at the experimental garden. Seeds were collected from Oil and Pulse Research Institute, Berhampore, West Bengal. Ten plants were kept in each 16 inches diameter pot and five sets were maintained as (1) Control, (2) NaCl stressed, (3) kinetin (10⁻⁵ M) pretreated NaCl stressed set, (4) kinetin (10⁻⁶ M) pretreated NaCl stressed set, (5) kinetin (10⁻⁷ M) pretreated NaCl stressed set. 20 pots were maintained for each treatment. Sets numbered (3), (4) and (5) were sprayed with kinetin (10⁻⁵ M), kinetin (10⁻⁶ M) and kinetin (10⁻⁷ M) solutions, respectively (50 ml per

pot), mixed with Tween-20 from 13 days (emergence of first trifoliate leaf) up to 35 days, once a week. Control set was sprayed with equal amount of water mixed with Tween-20. Then all these sets were treated with NaCl solution to maintain electrical conductivity (E.C) values of the soil as 4.0 m mhos/cm, 8.0 m mhos/cm and 12.0 m mhos/cm. The soil used was with electrical conductivity 0.3 m mhos/cm and pH 7.6. Electrical conductivity values were monitored on alternate days and adjusted if necessary. The set receiving no NaCl was designated as control. This condition was maintained until grain filling was complete. The garden temperature at the particular period when the experiment was conducted was $(34 \pm 2)^{\circ}\text{C}$. It was not artificially maintained.

At different maturation stages, (viz. pre-flowering, post-flowering and after grain filling) the penultimate leaves (second from the top) were collected to measure the amino acid and protein contents and to prepare a Gel electrophoretic profile of buffer soluble protein content in leaf. Amino acid content was estimated according to the ninhydrin method of Lee and Takahashi (1966), whereas buffer soluble protein content was estimated according to the method of Lowry *et al.* (1951). Gel was prepared following the Laemmli's (1970) method using SDS, which suits molecular weight determination of proteins. Separating gel was 12.5% and concentrating gel was 4.5%. The molecular weight marker used was Sigma : MW-SDS-200.

Results and Discussion

It was noted that of the three different electrical conductivity (E.C) values maintained by using NaCl solution, 4.0 m mhos/cm was the most effective sublethal concentration. It produced metabolic injuries to a moderate level, which could be restored to different degrees by the kinetin used. The experimental results at different maturation stages show that, stress protein appears at the post-flowering stage and persists until the completion of grain filling stage. Here, to co-relate the presence of stress protein during the grain

filling stage with protein and amino acid content within the leaf, the results obtained at this stage by only two treatments [viz. stressed set (E.C values 4.0 m mhos/cm) and kinetin pretreated stressed set] are presented here.

Salinity stress resulted in decreased amino acid content in mung bean leaf. There was 67% inhibition under stress, as compared with control. Kinetin was most effective at a concentration of 1×10^{-7} M, causing reduction in percent decrease from 67 to 21 (Fig. 2). Buffer soluble protein content decreased by 24% under stress. Kinetin at 1×10^{-6} M reduced the percent decrease from 24 to 8 (Fig. 3).

Salinity disturbs the accumulation pattern(s) of nitrogenous fraction, leading to a decrease in the total and protein nitrogen (Lal and Bhardwaj, 1987). It also induces hydrolysis of storage proteins by increasing protease activity, and decreases synthesis of free amino acids (Strogonov, 1962; Durgaprasad *et al.*, 1996). The uptake of nitrogen and the conversion of nitrate into organic nitrogen compounds become prone to disruption under salinity (Lapina, 1967).

Soluble protein content decreases with increased salinity level, but exogenous application of kinetin increases the soluble protein level (Fig. 3). It may be inferred that decrease in protein content is due to reduced capacity to incorporate amino acids into proteins and an increase in proteolytic enzyme or due to conversion of polysomes to monosomes under stress condition, or due to synthesis of ABA which accumulates during stress. ABA increases the activity of RNase, thus indirectly inhibiting the protein synthesis. Cytokinin production in roots and their translocation to the shoot is likely to be adversely affected under stress. Exogenous application of kinetin helps in maintaining the level of cytokinins which in turn help in increasing the incorporation of amino acids into proteins (Singh *et al.*, 1985).

Gel electrophoretic profile of buffer soluble protein content in leaf showed a remarkable change; an extra band between 29 kD

and 45 kD was present in stress protein profile. There was no change in protein profile in kinetin pretreated stressed plants (Fig. 1).

Several new proteins synthesized in plants in response to an altered environment have been reported as 'stress proteins' or 'shock proteins'

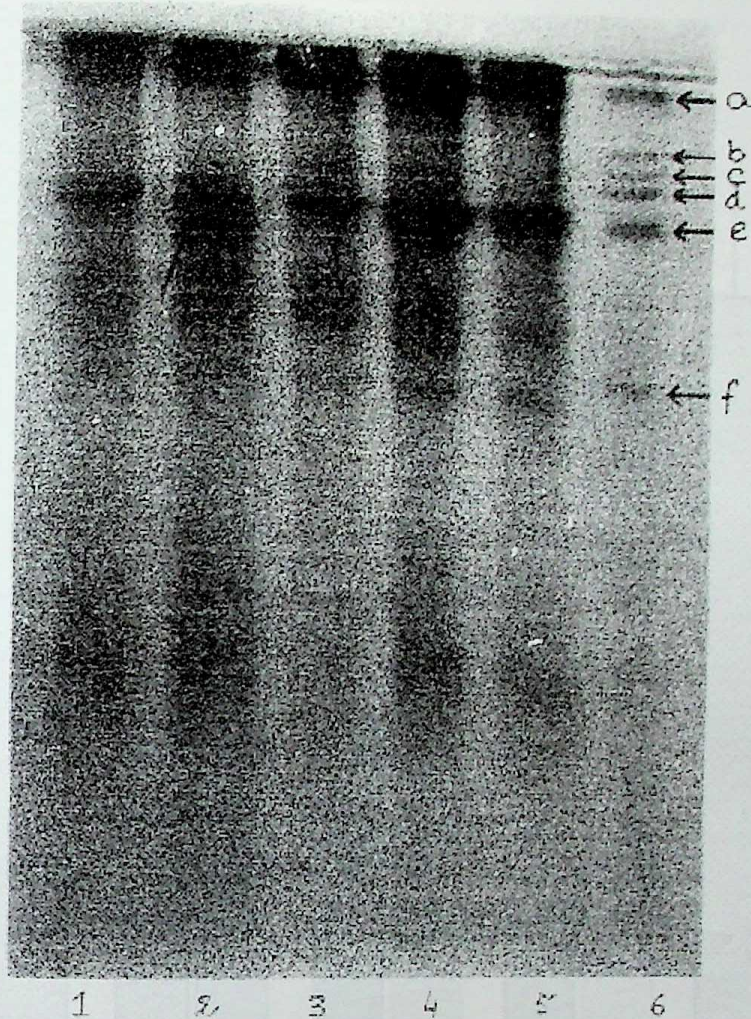


Fig. 1 : Gel electrophoretic profile of buffer soluble protein content in mung bean leaf -

- Lane - 1 : Control.
 Lane - 2 : NaCl Stressed (the arrow indicates an extra band in lane 2).
 Lane - 3 : Kinetin (10^{-5} M) pretreated NaCl stressed.
 Lane - 4 : Kinetin (10^{-6} M) pretreated NaCl stressed.
 Lane - 5 : Kinetin (10^{-7} M) pretreated NaCl stressed.
 Lane - 6 : Molecular weight marker proteins -
 a - 205 kD
 b - 116 kD
 c - 97.4 kD
 d - 66 kD
 e - 45 kD
 f - 29 kD

(Cooper and Ho, 1983; Kanabus *et al.*, 1984). Osmotic shock leads to the synthesis of new proteins and the extracellular release of several

proteins (Rubenstein, 1982). However, it is not clear how osmotic stress causes an alteration in the synthesis of specific proteins. Some of these shock

proteins are considered involved in possible transport functions (Amar and Reinhold, 1973; Rubenstein, 1982).

The salt-stressed proteins may possibly be involved in the adaptive process. The altered expression of genes for these proteins might be

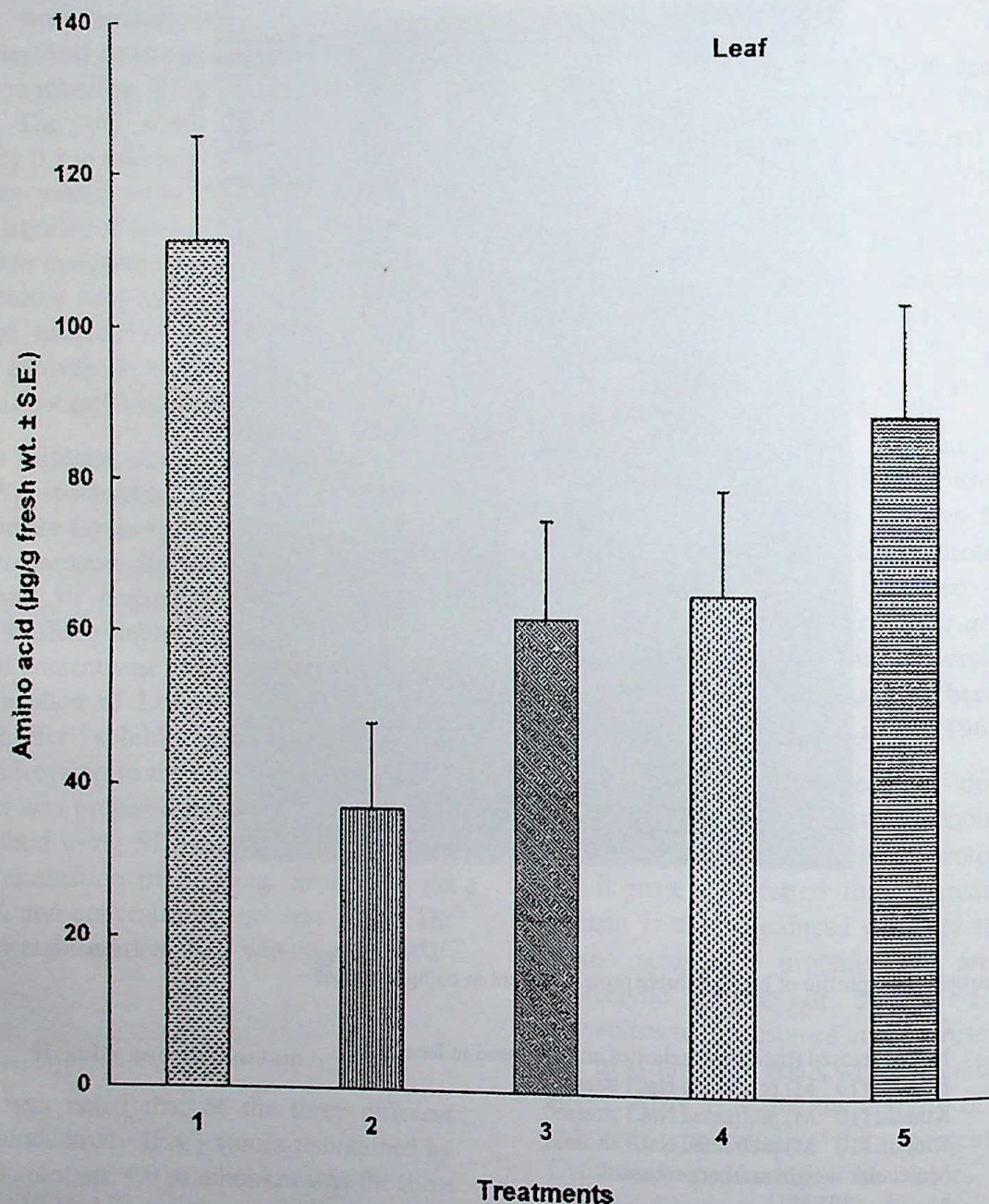


Fig. 2 : Vertical bars representing amino acid content of control and treated mung bean leaves, and showing standard error of the mean of 3 replicates ($P = 0.05$)

- | | | |
|---|---|--|
| 1 | = | Control set; |
| 2 | = | Stressed set; |
| 3 | = | Kinetin (10^{-5} M) pretreated stressed set |
| 4 | = | Kinetin (10^{-6} M) pretreated stressed set |
| 5 | = | Kinetin (10^{-7} M) pretreated stressed set |

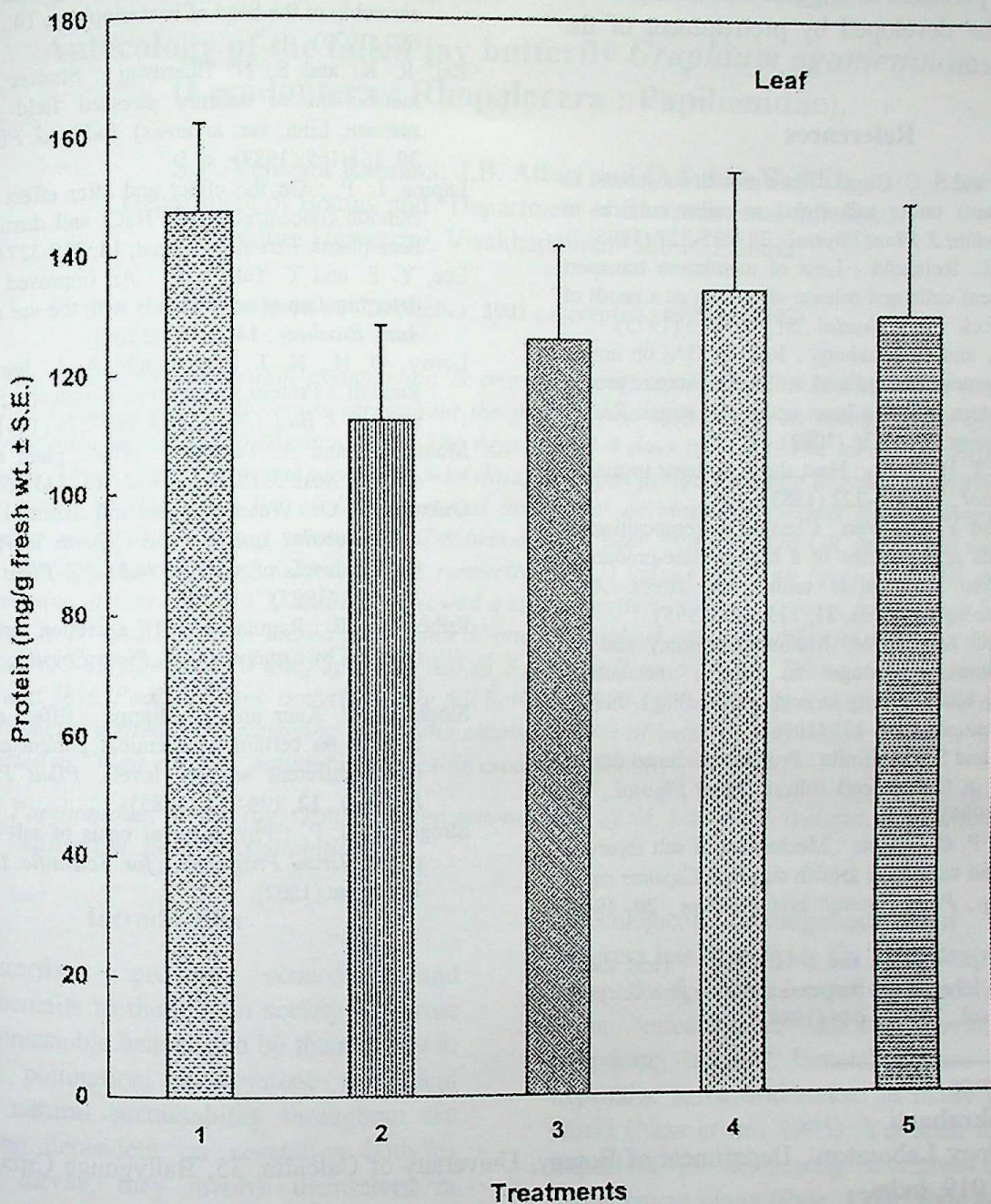


Fig. 3 : Vertical bars representing buffer soluble protein content of control and treated mung bean leaves, and showing standard error of the mean of 3 replicates ($P = 0.05$)

- 1 = Control set;
- 2 = Stressed set;
- 3 = Kinetin (10^{-5} M) pretreated stressed set
- 4 = Kinetin (10^{-6} M) pretreated stressed set
- 5 = Kinetin (10^{-7} M) pretreated stressed set

functionally involved in the ability of the cells to survive and grow in salt-containing medium (Ericson and Alfinito, 1984).

From the foregoing account, it appears that mung bean is suffering from alteration in protein and amino acid constituents induced by

salinity. It is pertinent to suggest that resistance to salinity can be developed by pretreatment of the crop with kinetin.

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Autecology of the tailed jay butterfly *Graphium agamemnon* (Lepidoptera : Rhopalocera : Papilionidae).

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Abstract : The Tailed Jay *Graphium agamemnon* is one of the attractive papilionid butterflies that enliven the environment of Visakhapatnam. It occurs throughout the year. It lays eggs singly on young leaves of the mast tree *Polyalthia longifolia* var. *pendula* (Annonaceae). The eggs take 3-4 days to hatch. The larvae go through 5 instars over a period of 15-16 days. The pupal period is 13-14 days. The total period from egg to adult emergence spans over 33-36 days. Based on this short life cycle, and larval and pupal development success studied every month, this butterfly can be multivoltine with a minimum of 7-8 broods in a year. Both CI and GR decreased with the age of larva, their average figures being 3.78 and 0.43 respectively. AD values are high (average 92%) and decreased through successive instars. Both ECD and ECI followed a similar pattern with an increase from instar I up to II, then a decrease up to IV and again an increase in instar V and the highest value is with fifth instar. Adults frequently visited flowers (12-35 flowers in a min) spending 1.0 to 3.2 seconds on a flower. The nectar concentration ranged between 16 and 58%. Peak foraging activity mostly fell between 0900-1000 h. The proboscis received pollen in most of the floral species visited, thus satisfying one of the characteristics of butterfly pollination. Being a fast and strong flier it is treated as "high energy" pollinator promoting cross-pollination.

Key words : Papilionidae, Tailed jay, *Graphium agamemnon*, Life cycle, Larvae, *Polyalthia*, Population index, Food utilisation, Butterfly pollination.

Introduction

Butterflies provide economic and ecological benefits to the human society by virtue of their incontestable beauty and by their ability to accomplish pollination, a keystone ecological process in natural sustainability throughout the world. Being dependent on vegetation both as adults and larvae, they involve themselves in complex feeding relationships with green plants. As adults they require a succession of adequate nectar resources and as larvae they are typically host specific. As such the butterflies provide the best rapid indication of habitat quality and also they are sensitive indicators of climate change. But the populations of such beneficial flagship insects are declining due to habitat loss and most of them are under the shadow of extinction (Varshney, 1986). In the temperate regions of the world there has been considerable progress in the conservation management of butterflies based on their

autecological investigations, but no similar progress has been made for many tropical species, except for few like *Ornithoptera* species. The fine-grain autecological studies carried out for temperate region butterflies are considered expensive to be undertaken in many parts of the world (New *et al.*, 1995). Yet such knowledge is important in developing effective conservation management plans (Pyle, 1976; Pyle *et al.*, 1981). For most Indian butterflies such autecological knowledge is woefully inadequate (Gay *et al.*, 1992; Gunathilagraj *et al.*, 1998). Sincere efforts are therefore being made in the Andhra University to remedying this deficiency for South Indian butterflies. Here we present the related information for the papilionid butterfly the Tailed Jay *Graphium agamemnon* Felder & Felder. This species with a wingspan of 85-100 mm is very showy due to brownish black upper side of wing surface with variable number of bright green spots

and stripes and enlivens the environment where its larval host plant, the mast tree *Polyalthia longifolia* var. *pendula*, is grown as an ornamental/avenue.

Materials and Methods

The Andhra University campus and the Zoo Park area 5 km away from the campus at Visakhapatnam (17°42'N-82°18'E) were regularly searched for the reproductive activity of the Tailed Jay butterfly *Graphium agamemnon* Felder & Felder during, 1998-1999. The Tailed Jay was found laying eggs on the mast tree *Polyalthia longifolia* var. *pendula* (Annonaceae). The eggs with the leaf material were brought to the laboratory and incubated, and further developmental stages were followed, and the success rates of egg hatching, larval and pupal development was also recorded. Young leaves were supplied daily to the growing larvae. Particulars of larval, pupal stages and the time of adult emergence were recorded from close observations. Searches were made every month for recording the different life stages-egg, larvae, pupae on 10 young plants of *P. longifolia* to work out the population index. Food consumption and growth at each instar stage were measured. Indices of food utilisation were calculated following Waldbauer (1968), and five replications were maintained for each index.

During the study period, the species of plants utilised as nectar resources and their flowering periods, corolla lengths, nectar volume and sugar concentration were recorded. Graduated micropipettes and refractometer were used to measure nectar volume and concentration respectively. Diurnal flower visiting activity pattern of adults on fine weather days was assessed by scoring the flowers visited in each hour from 0600 to 1800 h and the foraging speed by scoring the flowers visited in unit time and by determining the time spent at a flower in seeking nectar using a stop-watch. Pollen deposition on body parts was examined through light microscopy to assess pollination potential.

Results

Life History Stages : The Tailed Jay *Graphium agamemnon* lays eggs in singles on both surfaces of young leaves of *Polyalthia longifolia* var. *pendula*. It lays 8-12 eggs in a stretch but on different leaves of host tree. The eggs are spherical, surface smooth, 1.9-2.2 mm (2.0 ± 0.06) mm in diameter. They hatch 3-4 days after their deposition. The larvae go through five instars. Instar I lasts 2-3 days. It is 1.5-2.0 (1.8 ± 0.6) mm on day 1 and by day 2 it grows to 3.0-3.3 (3.1 ± 0.2) mm. Its thoracic region has hairy structures. Its body is snuff coloured, and abdomen is white. Head, abdomen and anal regions show snuff coloured spines. Instar II lasts 3 days. It is 4.5-5.5 (5.0 ± 0.14) mm in length. Its head is 1.5-2.0 (1.7 ± 0.06) mm in diameter. Its thorax is wider than other body parts. Instar III lasts 3-4 days. It grows up to 18-20 (19 ± 0.11) mm in length and 2-3 (2.6 ± 0.02) mm in width. Its head is 1.8-2.2 (2.0 ± 0.04) mm in diameter. Its body colour changes from snuff to pale brown. Its thorax is pale yellow with snuff coloured spines. Instar IV lasts 3-4 days. It is 32-34 (32.5 ± 0.11) mm in length and 3.5-4.5 (4.0 ± 0.12) mm in diameter. Body segmentation is clear with spots present on the body. Spines remain snuff coloured. Anterior part of the body is longer than posterior part. Instar V lasts 4-5 days. When fully grown it is green and 42-45 (43.5 ± 0.18) mm in length and 6.0-6.5 (6.0 ± 0.06) mm in width. Its head is 3-4 (3.5 ± 0.22) mm wide. Body segmentation is clear with spines size decreasing. There is no change in other characters. Fifth instar shortens itself by contraction within 2-3 days to enter pupal stage. It is 38-40 (39.5 ± 0.11) mm long. It attaches to the substratum with its entire body. This stage lasts 13-14 days. It is green, 28-30 (29.5 ± 0.17) mm long and 8.0-9.0 (8.3 ± 0.11) mm wide. Thorax bears snuff colour. Posterior end is pointed.

Larval Performance : The larvae first consumed their empty egg shell and later began to feed on leaf material. Feeding behaviour over a 4-h period in the laboratory revealed that the first 3 instars consumed food over 2-4 spells spending a total

time of 5-22 min, while the other 2 instars consumed in 4-6 spells spending a total time of 20-35 min. The first 3 instars ate slowly while the other two instars rapidly. There was a gradual

increase in the weight gain from instar to instar, the gain being low in the first 2 instars (0.0-2.2 mg) and thereafter it increased progressively through the remaining instars (Table 1).

Table - 1 : Feeding activity and growth of *G. agamemnon* larvae on *Polyalthia longifolia* leaves over a 4-h period.

Instar number	Day of observation	Number of feeding bouts	Total length of larval feeding time (min)	Wt. of larva (mg)		Wt. gain (mg)
				Initial	Final	
I	1	0	0	0	0	0
	2	2	5	1.02 ± 0.04	1.71 ± 0.05	0.69 ± 0.02
II	1	3	8	3.70 ± 0.07	4.60 ± 0.08	0.90 ± 0.06
	2	2	15	7.50 ± 0.10	8.60 ± 0.14	1.10 ± 0.08
	3	3	13	11.90 ± 0.17	14.10 ± 0.22	2.20 ± 0.11
III	1	3	14	21.20 ± 0.22	31.60 ± 0.26	10.40 ± 0.14
	2	3	20	89.40 ± 0.28	107.80 ± 0.32	20.00 ± 0.14
	3	4	22	160.20 ± 0.34	180.20 ± 0.44	20.00 ± 0.18
IV	1	4	20	267.80 ± 0.40	297.40 ± 0.48	29.60 ± 0.21
	2	5	22	352.20 ± 0.60	381.20 ± 1.00	29.00 ± 0.24
	3	5	27	401.50 ± 0.72	437.50 ± 1.28	36.00 ± 0.20
	4	4	26	489.50 ± 1.40	522.50 ± 1.64	33.00 ± 0.25
V	1	4	22	574.20 ± 2.05	613.20 ± 2.80	39.00 ± 0.28
	2	5	27	652.50 ± 3.30	697.50 ± 3.40	45.00 ± 0.29
	3	5	29	727.40 ± 4.85	773.00 ± 4.55	45.00 ± 0.32
	4	6	37	814.90 ± 6.20	871.50 ± 6.80	56.60 ± 0.38
	5	6	35	932.20 ± 8.20	994.50 ± 9.20	62.30 ± 0.42

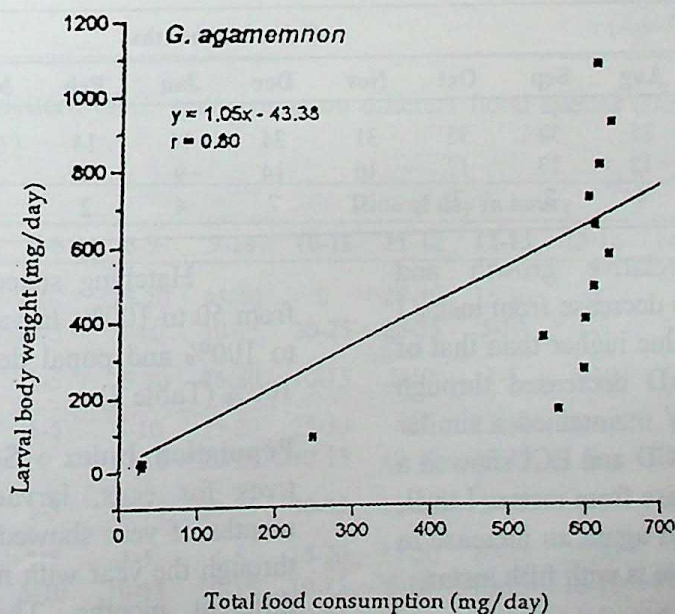


Fig. 1 : Larval growth as a function of food consumption rate in the *G. agamemnon*.

Quantitative data of food ingested, the faeces, weight gain by larvae, growth rate and consumption index are set out in Table 2. The

quantity of food consumed increased from instar to instar. There was threefold increase in instar II over instar I, 18 times more in instar III over instar

II, and about twice more in instar IV over instar III. Regression of weight gained by larvae against the food consumed per day showed a straight line

relationship between these two variables (Fig. 1), with r value ($r = 0.80$) greater than Table t value of 0.725 at 1% level.

Table - 2 : Food consumption and growth of *G. agamemnon* larvae on *Polyalthia longifolia*.

Instar No.	Wt. of food ingested (mg)	Wt. of faeces Larvae (mg)	Wt. gain by (mg)	GR	CI	AD (%)	ECI (%)	ECD (%)
1.	26.5 \pm 0.92	0.12 \pm 0.04	2.36 \pm 0.18	0.93	10.50	99	8.9	2.3
2.	78.3 \pm 2.10	3.08 \pm 0.240	13.5 \pm 0.67	0.43	2.50	96	17.2	17.9
3.	1413.0 \pm 7.20	109.50 \pm 2.90	206.3 \pm 3.70	0.55	3.78	92	14.6	15.8
4.	2390.5 \pm 12.40	186.4 \pm 3.20	267.4 \pm 3.90	0.16	1.48	92	11.2	12.1
5.	2733.5 \pm 13.60	486.5 \pm 4.10	518.8 \pm 4.50	0.12	0.65	82	18.8	22.9

Table - 3 : Hatching rate, pupal and adult development success of *G. agamemnon* in laboratory conditions.

Life stages	Calendar months											
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
# eggs incubated	8	15	24	18	20	18	8	6	6	4	6	5
# larvae hatched	7	14	23	18	16	14	6	3	4	2	4	3
# pupa formed	5	12	22	18	15	14	5	2	3	2	3	2
# adults emerged	5	12	22	17	15	14	5	2	3	2	3	2

Table - 4 : Population index of *G. agamemnon* on *Polyalthia longifolia* at Visakhapatnam.

Life stages	Calendar months											
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
# eggs	15	25	32	35	31	24	16	14	17	15	12	14
# larvae	5	12	13	12	16	14	9	7	6	4	5	7
# pupa	1	4	2	7	10	7	4	2	2	3	1	2

The values of relative growth and consumption index tended to decrease from instar I to V, but instar III had a value higher than that of instar II. The values of AD decreased through instars, but instar III and IV maintained a similar value. The values of both ECD and ECI showed a similar pattern with an increase from instars I to II, then a decrease up to IV and again an increase in instar V, and the highest value is with fifth instar.

Hatching, Pupal and Adult Development Success : The eggs collected in all months of year were incubated in the laboratory for observing the success of sequential stages of larvae, pupae and adults.

Hatching success during the year varied from 50 to 100%, larval development rate from 71 to 100% and pupal development rate from 94 to 100% (Table 3).

Population Index : Searches on 10 young host trees for eggs, larvae and pupae in different months of year showed that these stages occur all through the year with numerical variation between different months. The life stages were more frequent during August-December (Table 4).

Adult Nectar Resources : Based on field observations, 19 different plant species were found to be frequently visited for nectar. Flowering periods of these plants were noted (Table 5), and

nectar concentration was assessed (Table 7). Diurnal foraging on different floral species, number of flowers covered in a minute and time

spent (seconds) at a flower were also recorded (Table 6; Table 7). The butterfly visited 12-35 flowers in a minute spending 1.0 to 3.2 seconds on

Table - 5 : Floral resources of *G. agamemnon* at Visakhapatnam.

Floral species	Flowering period
<i>Albizia lebbeck</i>	Mar-May
<i>Anacardium occidentale</i>	Dec-Mar
<i>Antigonon leptopus</i>	Throughout year (Sep-Apr)
<i>Bougainvillea spectabilis</i>	Major part of year
<i>Caesalpinia coriaria</i>	July-Sep
<i>Caesalpinia pulcherrima</i>	Throughout year (Feb-Jul)
<i>Catharanthus roseus</i>	Throughout year (Sep-Mar)
<i>Citheroxylon subserratum</i>	Apr, Jul & Sep
<i>Duranta repens</i>	Jun-Dec
<i>Enterolobium saman</i>	Mar-May
<i>Jasminum angustifolium</i>	Jun-Aug
<i>Jatropha podagrica</i>	Throughout year
<i>Lantana camara</i>	Throughout year
<i>Moringa oleifera</i>	Mar-May, Aug-Sep
<i>Murraya koenigii</i>	Apr-May
<i>Sapindus emarginatus</i>	oct-Feb
<i>Sida cordifolia</i>	Aug-Dec
<i>Tectona grandis</i>	Jun-Aug
<i>Vitex negunda</i>	Throughout year (Apr-Jun)

Table - 6 : Diurnal activity pattern of *G. agamemnon* on different floral species (Data for each hour is given as percentage of total daily activity).

Floral species	Time of day in hours											
	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18
<i>Jatropha podagrica</i>	0	0	15-20	35-40	0	25-30	15-10	0	0	0	0	0
<i>Murraya koenigii</i>	1-5	1-5	5-10	10-15	20-25	20-25	5-10	1-5	5-10	1-5	1-5	1-5
<i>Caesalpinia pulcherrima</i>	0	1-5	25-30	25-30	10-15	5-10	1-5	5-10	5-10	0	0	0
<i>Albizia lebbeck</i>	0	1-5	5-10	15-20	25-30	0	25-30	10-15	1-5	1-5	0	0
<i>Catharanthus roseus</i>	0	0	10-15	20-25	10-15	10-15	10-15	0	10-15	5-10	0	1-5
<i>Anacardium occidentale</i>	0	1-5	10-15	20-25	30-35	15-20	5-10	1-5	1-5	0	0	0
<i>Duranta repens</i>	0	1-5	1-5	1-5	15-20	15-20	20-25	25-30	0	0	0	0
<i>Vitex negundo</i>	0	5-10	10-15	10-15	10-15	5-10	10-15	10-15	15-20	5-10	1-5	0
<i>Caesalpinia coriaria</i>	0	0	1	40-45	20-25	1-5	20-25	1-5	5-10	0	0	0
<i>Sapindus emarginatus</i>	0	1-5	5-10	35-40	15-20	10-15	5-10	1-5	1-5	1-5	0	0

a flower in sucking nectar. Nectar concentration of floral species varied from 16 to 58%. The butterfly foraged during daylight hours and the peak

foraging time varied with the floral species, but mostly fell between 0900-1000 h (Table 6). Proboscis length of *G. agamemnon* approximated

to 25 mm, and corolla tube length varied between 4-39 mm, the average length being 12.4 mm. While nectaring, the butterfly hovered with wings fluttering, and various body parts like proboscis, antennae, head, legs and wings came into contact

with essential organs of the flower, and the actual part that contacted essential organs depended on floral architecture. Out of the 13 plants investigated, the pollen of 12 plants got deposited on the proboscis, 4 on head, 3 on wings, 2 on legs

Table - 7 : Nectar characteristics of the floral and foraging speed of *G. agamemnon*.

Floral species	Time spent per each flower (Sec)	No. of flowers visited per 1 min	Nectar volume		Nectar concentration (%)	
			10.00 h	17.00 h	10.00 h	17.00 h
<i>Caesalpinia pulcherrima</i>	2.3	23	0.68	1.22	29.00	18.00
<i>Duranta repens</i>	2.3	25	0.60	1.00	24.00	19.00
<i>Caesalpinia coriaria</i>	3.0	18	0.02	0.06	48.00	26.00
<i>Sapindus emarginatus</i>	1.5	16	1.30	1.60	39.00	26.00
<i>Murraya koenigii</i>	2.0	26	0.06	0.01	23.00	16.00
<i>Sida cordifolia</i>	2.0	23		Traces-not quantifiable		
<i>Jatropha podagrica</i>	2.1	14	1.40	1.80	56.00	53.00
<i>Antigonon leptopus</i>	2.3	19	0.02	0.40	58.00	54.00
<i>Albizia lebbeck</i>	3.2	35	0.40	0.70	19.00	17.00
<i>Enterolobium saman</i>	2.5	12		Traces-not quantifiable		

and one plant on antennae. Similarly, the frequency of contact of various body parts with stigma varied. Thus it is mostly the proboscis that gained contacts with essential organs in most floral species and mediated pollination.

Discussion

Most Papilionids lay their eggs singly (Stamp, 1980), and the tailed jay *Graphium agamemnon* is one among them. A general constraint on egg-laying habit is the amount of food offered by a host plant. Then those butterflies with single egg-laying habit are expected to have the small plants as hosts. But *Polyalthia longifolia* var. *pendula* and all other host plants of *G. agamemnon* represent a tree habit. Such tree habit always with abundant foliage offers an advantage to this butterfly species in that the larvae are less likely to be exposed to environmental conditions of heat and desiccation.

The eggs took 3-4 days to hatch. The five larval stages took 15-16 days to develop up to the prepupal stage. The final instar took 2-3 days for prepupation to enter the pupal stage. The pupa took 13-14 days to transform into the adult. Thus

the total period from the egg stage to adult emergence is computed to span over 33-36 days. This short life cycle period, population index and laboratory records of egg, larval and pupal development success suggest the tailed jay is multivoltine. Assuming an adult life of 7-12 days (Opler and Krizek, 1984), the number of broods that may occur in a year comes to a minimum of 7-8. The behaviour of *G. agamemnon* with its year round distribution showing up better during August-December is in line with the prediction of Owen (1971) that tropical butterflies breed all through the year doing better in certain period of the year. However, *Graphium doson* in the same biotope has a seasonal distribution, from April to November and doing better during May-August. Probably these two related species have different life style adaptations to climatic conditions, and a study of the same is worth undertaking.

Over the entire period of its growth, a larva on an average consumed over 6.6 gm of leaf material. Instar wise consumption increased with the advancing age, the last two instars consuming over 77% of the total food consumed. This tendency of greater consumption by the last two

instars has been reported in lepidopterous larva in general (Waldbauer, 1968; Mathavan and Pandian, 1975; Scriber and Slansky, 1981; Palanichamy *et al.*, 1982; Selvasundaram, 1992; Gosh and Gonchaudhuri, 1996), and it compensates the energy expenditure of non-feeding pupal stage (Pandian, 1973). This increasing food consumption at successive instars is in inverse relationship with consumption index (CI) and growth rate (GR). Both CI and GR decreased with the age of larva, the former from a high of 10.50 to a low of 0.65 and the later from a high of 0.93 to a low of 0.12. The average figures of these two indices are : CI = 3.78; GR = 0.43. The values of CI are within the range (0.31-6.60) predicted for tree foliage chewers (Slansky and Scriber, 1985), and they correspond with the values (0.92-3.31) estimated for the swallowtail butterflies (Scriber and Feeny, 1979). Food consumption rate depends on the conversion efficiency of ingested food to biomass (ECI), the rate increasing as the conversion efficiency decreases or vice versa (Slansky and Scriber, 1985) and the high CI value (10.50) of instar I is probably due to low conversion efficiency and this character is reflected in the low value of ECI for instar I compared to other successive instars. Higher growth rates occur with penultimate than with final instar (Scriber and Feeny, 1979). The GRs of penultimate and final instar of *G. agamemnon* are in line with the above decreasing trend.

The approximate digestibility (AD) values decreased from instar I to instar V, the highest value being associated with instar I (99%) when the food intake of the larva is 0.29% of total consumption and the lowest value with the instar V (82%) when the food intake of this instar is 41.15%. These values are comparable with the range of AD values (19-81%) for lepidopterous larvae (Pandian and Marian, 1986) and for *Pericallia ricini* (28.7-84.6%). The average AD percentage is over 92 and this high AD substantiate the statement of Slansky and Scriber (1985) that foliage chewers often attain high AD values. Such high AD values also are expected when food item is rich in nitrogen (and also water)

(Pandian and Marian, 1986). Similar results were repeated with *Pieris brassicae* (Yadava *et al.*, 1979), and *Euploea core* (Venkata Ramana *et al.*, 2001).

The values of ECD increase from early to late instars (Slansky and Scriber, 1985). Though such trend is broadly apparent with the ECDs of *G. agamemnon*, with the lowest value in instar I and the highest in instar V, there is no progressive increase across the instars. Literary data also show no uniform trend, some reporting a rise up to instar IV (Yadava *et al.*, 1979; Rana *et al.*, 1987; Barah *et al.*, 1989), and some recording a gradual increase across the instars (Gupta and Vats, 1980). The ECDs obtained are low compared to the ADs and such low values are not unusual (Waldbauer, 1968). This is indicative of low efficiency of conversion of digested food to body tissues. This poor utilisation of food is often attributed to deficiency in some essential nutrient in food (Bailey and Mukherji, 1976), or a factor causing an increase in energy expenditure on metabolism (Muthukrishnan, 1990). The pattern of ECI values followed quite closely the pattern of ECD. The values (8.9-18.8%) obtained are comparable with the range of values expected for foliage chewers (2-31%) (Slansky and Scriber, 1985) and for the swallowtail butterflies (6.7-41.5%) (Scriber and Feeny, 1979). Although the pattern of ECI is expected to follow that of AD (Waldbauer, 1968), the two indices of *G. agamemnon* have no similarity and there is no definite trend of increase or decrease in ECI values, thus substantiating the inconsistent trend in ECI values suggested by Slansky and Scriber (1985).

Floral nectar is an important food resource for butterflies (Boggs, 1987). *Graphium agamemnon* visits flowers frequently and imbibes nectar (also Bell, 1912; Larsen, 1987), and nectar intake may increase its longevity and egg production (Stern and Smith, 1960; Murphy *et al.*, 1983). The most effective nectar sugar concentration is 40% in *Thymelicus lineola* (Pivnick and McNeill 1985) and in *Papilio xuthus* it is 50% for egg maturation (Watanabe, 1992). The

sugar concentration of *G. agamemnon* flowers ranged between 16-58% and it is comparable with the range (15-50%) of psychophilic flowers (Kevan, 1997). In its visits to flowers, *G. agamemnon* receives pollen mostly on proboscis, and deposition of pollen on proboscis (and head) is one of the pollination traits of butterflies (Kevan, 1997). Being a fast and strong flier, and hovering (and fluttering) while taking nectar, *G. agamemnon* perhaps requires more energy and thus visits a large number of flowers in unit time, and such "high energy" pollinators may cover relatively long distances and promote cross-pollination (sensu Heinrich and Raven, 1972; Heinrich, 1975).

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Hepato and nephrotoxicity in rat exposed to endosulfan.

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Abstract : The indiscriminate and injudicious use of pesticides particularly endosulfan in agriculture and animal husbandry practices has considerably increased the risk of human health hazard. The present work was therefore undertaken to evaluate the toxic effect of endosulfan on the vital organs viz. liver and kidney of rat. Oral administration of endosulfan at the dose level of 10 mg/kg b.wt./day for two and four weeks showed toxic interference with the biochemistry and histology of rat liver and kidney. The biochemical parameters viz. Aspartate amino transferase, alanine amino transferase, acid phosphatase, alkaline phosphatase, bilirubin urea and creatinine were increased which clearly showed the hepato and nephrotoxic effect of endosulfan. Histopathologically the size of liver was increased, sinusoidal dilation, pyknotic nuclei, cytoplasmic degranulation and various nuclear aberrations were observed. Similarly pathological alterations viz. chronic glomerulonephritis, glomerulosclerosis, odenoma and glomerulus deposits were observed in the kidney.

Key words : Endosulfan, Biochemistry, Histology, Liver, Kidney, Rat.

Introduction

Endosulfan (WHO, 1984) (Thiodan 6, 7, 8, 9, 10, 10-hexachloro 1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano- 2, 4, 3-benzodioxathiepin-3-oxide) a broad-spectrum cyclodiene pesticide of organochlorine group is widely used in agriculture for the control of pests. It is one of the highly toxic organochlorine insecticides. Because of its longer persistent and slower degradation, many non-target animals including human beings get affected. The studies have been conducted on acute toxicity of endosulfan. Oral administration of endosulfan caused increase in weight of liver and lungs and decrease in weight of testes in male rats (Ansari *et al.*, 1984). Naqvi and Vaishnavi (1993) found that endosulfan exposure caused renal, hematological and hepatotoxicity in rats. It also caused testicular atrophy and ovarian cysts in rats and reduced the weight of secondary sex organs. However, the literature on exposure of endosulfan on haemopoietic system and serum analysis, specifically in mammals is scanty. Therefore, an attempt has been made to study the influence of chronic administration of endosulfan on histology of liver and kidney and serum biochemistry in rats.

Materials and Methods

Animals : The albino rats of Sprague-Dawley strain (*Rattus norvegicus*) obtained from Jamia Hamdard University, New Delhi were housed in plastic cages at room temperature ($20 \pm 5^\circ\text{C}$) and uniform light (14 : 10 : : L : D). They were fed on standard laboratory chow (Ashirwad Food Industries Ltd., Chandigarh, India) and fresh water *ad libitum*.

Pesticide : Technical endosulfan (α and β isomers in the ratio of 70 : 30) was commercially obtained from Hoechst, Bombay (India) was used for the experimentation.

Treatment : Healthy rats weighing 150-200 gm were divided into three groups of five animals each. The control group I received only the vehicle groundnut oil, whereas the animals of group II and III were administered, endosulfan dissolved in groundnut oil orally by pearl point needle at the dose level of 10 mg/kg. b.wt./day for 15 and 30 days respectively.

Experimental procedure : The animals were weighed and autopsied under light ether anesthesia and the blood from heart was collected in

preheparinized tubes. The serum was obtained by centrifugation at 3000 rpm.

Biochemical estimations of serum were done colorimetrically by the following standard methods. Alanine amino transferase and Aspartate amino transferase were assayed by the Reitman and Frankel's method (Reitman *et al.*, 1970; Balazs *et al.*, 1962), serum acid and alkaline phosphatase were estimated by King and King's method (Kind and Jagathesan, 1959), bilirubin was determined by the modified Jendrassik and Grof method (Jendrassik *et al.*, 1958). Urea was estimated by Berthelot method and creatinine was determined by commercially available kit of Ranbaxy Laboratory Ltd.

The vital organs viz. liver and kidney were taken out, free of fat and weighed separately on electronic balance, they were fixed in Bouin's fixative, cut into pieces and processed through ethano-xylene series, embedded in paraffin and bee wax (3 : 1), sections were cut at 5 μ and stained with hematoxyline and eosin. The data were analyzed statistically by using students 't' test.

Results and Discussion

The results of the present study revealed that the chronic exposure of endosulfan produces significant changes in the liver and kidney histology as well as in serum biochemical parameters of rat in a dose dependent manner. The

Table - 1 : Alterations in serum biochemistry after endosulfan treatment in rats.

S. No.	Parameters	Control	10 mg/kg b.wt./day	
			15 days	30 days
1	Aspartate amino transferase (Units/ml)	130.18 ± 0.02	148.75 ^{ns} ± 6.00	158.75 ^{ns} ± 10.35
2	Alanine amino transferase (Units/ml)	72.20 ± 1.90	112.5*** ± 6.23	125.0*** ± 2.34
3	Serum acid phosphatase (KA units)	4.59 ± 0.08	4.95* ± 0.09	5.87 ^{ns} ± 1.11
4	Serum alkaline phosphatase (KA units)	30.0 ± 0.5	5.6 ^{ns} ± 2.6	5.8 ^{ns} ± 1.3
5	Bilirubin (mg%)	0.20 ± 0.00	0.73** ± 0.09	0.72 ^{ns} ± 0.31
6	Urea (mg/dl)	48.52 ± 3.70	74.00** ± 2.56	89.56*** ± 1.61
7	Creatinine (mg/dl)	1.22 ± 0.03	2.30** ± 0.05	2.80** ± 0.04

Values given are Mean \pm SEM of results obtained from five animals.

ns = non-significant;

*

= Significant ($P \leq 0.05$);

= Significant ($P \leq 0.001$).

**

= Significant ($P \leq 0.01$)

increase in hepatic acid and alkaline phosphatase activity in intoxicated animals, observed in the present investigation may be due to the destruction of lysosomal membrane which resulted in the release of enzymes or failure of secretory functions of liver cell (Galdhar *et al.*, 1998; Sharma and Sastry, 1998; Saxena and Sarin, 1980). Similar increase in the aspartate and alanine amino

transferase indicates the hepatotoxic action of endosulfan due to cellular damage (Drotman and Lawhorn, 1978; Gillette and Keppler, 1975; Molander *et al.*, 1955; Siddiqui *et al.*, 1987; Singh and Pandey, 1991), which includes destruction of hepatocytes, hyperplasia and hypertrophy of islet cells (Amminikutty and Rege, 1977). The elevation of serum bilirubin concentration indicates

Hepato and nephrotoxicity of endosulfan.

possibility of severe parenchymal injury (Braurer, 1959; Klaassen, 1977; Plaa and Hewitt, 1982). Further, the hyper creatinemia is considered a

potential end point of renal toxicity with decrease in glomerular filtration rate (Goldstan and Schnellmann, 1996). Similarly, increased urea also

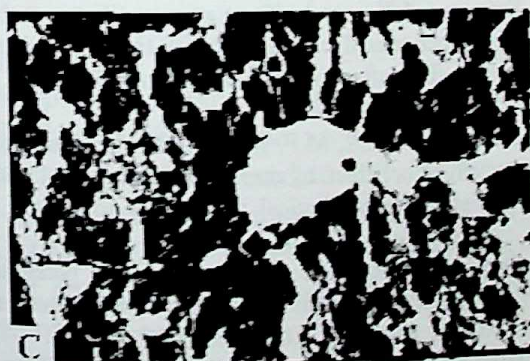
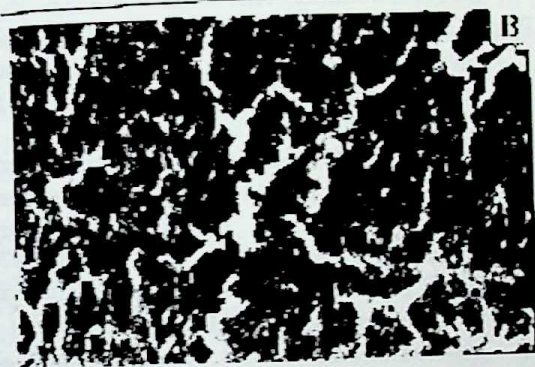
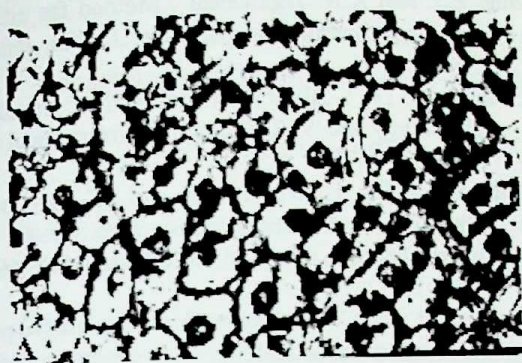


Fig. A : Photomicrograph of control rat liver showing typical morphological architecture. H & E 500X; B : After 15 days endosulfan exposure, large number of small round bodies stained a deep purplish black are present within the hepatocytes. (thick arrow). Dilation of sinusoidal spaces and star shaped nuclei are seen. H & E 100X; C : 30 days exposure of endosulfan shows many degenerative changes marked by binucleated cells, hypertrophy in hepatocytes, nuclear aberration and lymphocytic infiltration in the central vein. H & E 200 X; D : Photomicrograph of control rat kidney showing typical morphological architecture. H & E 50 X; E : After 15 days endosulfan exposure the epithelial cells are replaced by flattened cells. Few of the epithelial cells are vacuolated and necrotic. The ateriole (arrow) has thick hyalinized wall and a much-reduced lumen. H & E 100X; F : After 30 days endosulfan exposure there is a loss of glomerulus and many of those remaining (e.g. side and bottom) are small and atrophic. One glomerulus is sclerosed with greatly reduced cellularity and is adherent to Bowman's capsule. H & E 50X.

showed renal toxicity due to increased protein catabolism (Table). The above observations were supported by definite histological changes; the size of the liver was increased (hypertrophy) along with

the dilation of sinusoidal spaces (Fig-B). The nuclei also showed varied appearance some were darkly stained while other hepatocytes were binucleated and multinucleated, cytoplasmic

degranulation with various nuclear aberrations such as karyolysis; karyorrhexis, karyoschisis and pyknosis were reported (Bhatnagar *et al.*, 1987) (Fig-c). Similarly, pathological alterations were noticed in the kidney (Bhattacharya and Mukherjee, 1975), which includes replacement of epithelial cells by flattened cells chronic glomerulonephritis (Fig-E), glomerulosclerosis, odenoma, pyknotic nuclei and glomerulus deposits (Fig-F). Thus in the present study endosulfan induces intoxication on the structure and function of liver and kidney.

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Physicochemical and microbiological assessment of Oko-oba – A Nigerian Abattoir.

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Abstract : The physicochemical and microbiological assessments of Oko-oba abattoir were carried out during dry and wet season to determine whether the waste discharges are within tolerable limits.

All the physicochemical parameters studied showed seasonal variations. Higher temperature and lower pH values were recorded during the dry season than wet season. Similarly, the BOD of wastewater from the slab decreases from 10000 mg/ml during the dry season to 6000 mg/ml in the wet season. Conversely, the BOD of the final waste discharge was 4000 mg/ml during the dry season and 3,500 mg/ml during the wet season. The COD showed similar trend, with values ranging from 12,200 to 18,500 mg/ml depending on the season. The suspended solid values ranged from 1200 to 1950 mg/ml. The phosphate and nitrate ions were in the range of 41-75mg/l and 22.5- 960 mg/ml respectively. Heavy metals detected were Chromium at 104 -114 mg/ml, Copper 16 -75 mg/ml and Iron 55- 114 mg/ml.

The following bacteria species were also encountered :

B. cereus, *E. coli*, *P. aeruginosa*, *S. aureus*, *S. faecalis*, *S. lactis*, *Micrococcus* sp, *A. aerogenes*, *P. vulgaris*, and *S. typhi*. The results showed significantly high level of pollutants in the waste discharged.

Key words: Physicochemical, Microbiological, Oko-oba lairage, Pollution.

Introduction

An abattoir is an establishment where cattle and other livestock such as goats, lambs, sheep, pigs etc., are slaughtered to provide meat and other edible parts for human consumption (Scarafoni, 1956). Lagos State (Nigeria) with a population of about 12 million has only one large government owned abattoir at Oko-oba in the suburb of Agege Local Government Area, although there are several privately owned smaller abattoirs within the metropolis and suburb.

Discharge from abattoirs and slaughterhouses fall among the group of industrial wastes. Its wastewater consists mainly of water and suspended solids (sand particles and meat/bone bits), soluble organic and inorganic ions (Ten Hare, 1976). It also contains high amount of nutrients therefore aiding the growth of microorganisms such as bacteria, viruses, protozoa and intestinal parasites which have been known to

cause enteric infections in man (Okafor, 1985). Abattoir waste primary treatment involves screening, sedimentation, chemical precipitation, trickling filters and activated sludge processes (Targanides *et al.*, 1979). Screening removes coarse materials such as hair, flesh, paunch and floating solid by rotatory wire mesh. Sedimentation removes 63% of suspended solid, 35% of BOD, trickling filters removes between 81 and 90% BOD with no accompany nuisance (Black and Brown, 1974). Blood is separated and all suspended solids are reduced by addition of alum. Fat is extracted by introducing a preheating salient, which unite with the fat in the material to form a concentration miscella, removed by settling tanks and passed to an evaporator.

Aerobic bacteria breakdown the organic material when there is sufficient supply of oxygen available. Ammonia is converted to nitrite; mineralization increases due to volatile solids and aerobic treatment removes biodegradable organic

matters from wastewater (Burchbaker *et al.*, 1971). All these procedures are not practiced at Oke-oba abattoir. Indeed most operations are carried out by illiterate butchers under largely unhygienic conditions and the resultant waste waters are not treated according to the standard procedures.

This study was embarked upon to assess the microbiological and physicochemical qualities of the wastewater discharged at Oke-oba with a view to determining the level of pollution being discharged into the environment and to make recommendations.

Materials and Methods

Study area : The Lagos State abattoir lairage is at Ifako-Ijaye local government area in Lagos. Topographically, the lairage is on lowland area with vegetation of little grassland adjacent to the abattoir. The abattoir is a standard one containing big lairages, cattle pens, refuse houses, drainage ditch, bore holes, municipal water taps, meat inspection laboratory and a slaughtering house which has been replaced with a slaughtering slab due to lack of ability to maintain the sophisticated slaughtering equipments.

Slaughtering procedure : Cows are rested at the lairage before slaughtering process commenced. All waste including blood, paunch, hide, dung, fat and dirt are virtually washed down as wastewater, which undergo minor treatment before being discharged.

Sampling : Samples were collected at two sites within the months of November and December 1998 (representing dry season) and May and June 1999 (representing wet season)

Sample A : 100ml of run off from the slaughtering slab was aseptically collected into a presterilized 500 ml amber jars.

Sample B : 100 ml of waste water from the abattoir lairage discharging into the municipal sewer was aseptically collected in presterilize 500ml amber jars.

The samples were then taken straight to FEPA laboratory for microbiological and physicochemical analysis.

Physicochemical analysis : The following physicochemical parameters were determined using standard methods (APHA, 1972).

Temperature, hydrogen ion concentration (pH), suspended solids, colour, conductivity, turbidity, total dissolved solid, Chemical oxygen demand (COD), Biochemical oxygen demand (BOD), The concentrations of the following ions were also determined : phosphate, nitrate, nitrite sulphite, sulphide, and chlorine.

Finally, the concentration of the following heavy metals was determined : Iron, Chromium and Copper.

Microbiological analysis : Samples were plated on the following media within two hours of collection. Plate count Agar (for total bacterial count) McConkey agar (for *E. coli*, *Salmonella* sp and *Shigella*) S-S-Agar (for *Salmonella* -*Shigella*) L-Eosine Methylene Blue agar (for *E. coli*) and PDA (for fungal and yeast).

Serial dilution of each sample was carried out in test tubes using sterile distilled water. 1 ml of 10^6 dilutions was plated (using the pour plate method) into the appropriate media. The plates were incubated at 37°C for 24 hours for bacterial isolates and 72 hours for fungal isolates.

Thereafter colonies appearing on plates were counted and purified by subculturing on fresh agar plates.

The organisms were later identified using standard methods (Cowan and Steel, 1974).

Results and Discussion

The result of the physicochemical analysis is shown in Table 1.

Higher values were recorded in sample B in the following parameters during the dry season : pH, conductivity, dissolved solids, and Nitrate ion while Sample A recorded higher values in the following parameters : Colour, Temperature

Suspended solid Turbidity, Phosphate, Sulphate, Sulphite, Chlorine, BOD, COD, Iron, Chromium and Copper.

Similarly during the wet season, sample A recorded higher values in suspended solids, turbidity, and most other parameters except phosphate ion concentration and pH.

Comparative analysis of the two seasons was recorded. The following parameters Colour, Temperature, suspended solid, Turbidity, Dissolved solid, Nitrate, Phosphate, Sulphate, BOD, COD, Iron, Copper and Chloride ions were recorded for dry seasons. The wet season recorded higher values in pH, sulphite, and chromium ion.

Table - 1 : Physicochemical analysis of effluent from Oko-oba Lairage during dry and wet season. (Each figure is an average of 3 readings).

	Month/time			
	Oct.	Dec.98	May	July 1999
Samples	Sample A	Sample B	Sample A	Sample B
Colour	9.850	3800	5,206	3,100
pH	6.70	6.82	7.17	7.21
Temp ($^{\circ}$ C)	35.5	34.5	28	26
Suspended solid (mg/l)	1950	1900	1600	1200
Conductivity (ms/cm)	4.08	14.83	10	9
Turbidity (FTU)	7075	4750	5,200	3,750
Dissolved solid (mg/l)	2040	7410	1,500	1,800
Nitrate (mg/l)	600	960	33	22.5
Phosphate (mg/l)	75.9	49.75	24	41
Sulphate (mg/l)	1900	1300	420	250
Sulphite (mg/l)	5.78	4.58	13.8	9.50
Chlorine (mg/l)	740	280	179	98
BOD (mg/l)	10000	4000	6,000	3,500
COD (mg/l)	18,500	15,000	14,200	12,200
Iron (mg/l)	114	40	78	55
Chromium (pt co)	114	12.4	120	104
Copper	75.4	57.6	58	16

A=Waste from slaughter slab

B=Final effluent discharge.

The result of the microbiological analysis is shown in Table 2. The following bacterial species were identified :

B. cerus, *P. aeruginosa*, *S. aureus*, *S. faecalis*, *S. lactis*, *Micrococcus* sp, *K. aerogenes*, *P. vulgaris*, *E. aerogenes*, *S. typhi*.

The fungal isolates encountered were *A. niger*, *A. fumigatus*, *Penicilium* sp, *Mucor* and *Rhizopus* sp.

There was not much difference in the bacteria load of the waste from slaughtering slab (Sample A) and the waste discharging out (effluent) (Sample B). However higher fungal

count was recorded in the dry season than wet season.

The slaughterhouse wastes and the effluents were observed to have the characteristics reddish brown colour, which was obviously due to presence of blood. This is characteristic of slaughterhouse waste (Black and Brown, 1974). The foul smell which pervaded the whole environment was obviously due to malodorous gasses produced during the anaerobic decomposition of the waste that brought about liberation of such gasses like Ammonia, Hydrogen Sulphide, methane, etc.

Expectedly high figures were recorded in the parameters screened with the slaughterhouse wastes higher than the effluent because the later had undergone some mild treatment. The high BOD values recorded is an indication of high microbial activities resulting in high demand for

available dissolved oxygen. Similar reasons could be adduced for the high values of the COD. Generally, high BOD and COD is typical characteristics of abattoir waste and it indicates a very high risk if such wastes are not properly treated before discharge (Griffiths, 1981).

Table - 2 : Distribution of the various groups of aerobic microorganisms in effluents discharge from Oko-oba lairage (Each figure represents an average of 3 readings).

Sample source	Mean total heterotrophs bacteria (cfu/ml)	Mean total coliform and pathogenic bacteria (cfu/ml)	Mean total fungi (cfu/ml)	Month
Sample A	2.0×10^8	2.5×10^7	5.0×10^3	Oct to Dec 1998
Sample B	2.2×10^8	2.0×10^7	6.0×10^3	
Sample A	1.5×10^8	2.2×10^7	3.0×10^3	May to July 1999
Sample B	1.2×10^8	1.6×10^7	3.0×10^3	

A=Waste from slaughter slab

B=Final effluent discharge.

When results obtained for the two seasons are compared it will be seen that a general decrease for dissolved inorganic ions as well as the pH in wet season. This can be attributed to flooding or availability of more water therefore more of these ions got dissolved (Odiete, 1999). The high sulphate contents observed are potentially hazardous because it can lead to increase in sulphide content of manure from livestock as well as in the environment (Dunham *et al.*, 1985). The phosphate, nitrate and other ions are potential source of an "algal bloom" in receiving waters

flora and intestinal content (Frazier and Westhoff, 1988). No seasonal variation was noticed in the number of these bacteria that got into the waste. However, the fungi counts were higher in the dry season obviously due to the presence of many air-borne spores in the environment.

Overall, it was seen that the abattoir sites do not conform to the FEPA (Federal Environmental Protection Agency) standard (FEPA, 1991). Almost all parameters screened exceeded the recommended limits by very wide margins. Thus, these discharges are quite harmful to the receiving body of water and subsequently to the exposed communities. It is thus recommended that waste be treated and converted to useful means. For example, in Europe, solid and liquid wastes from animal feed are treated appropriately. The refined wastes serve as manures to promote plant growth due to its high nitrate and phosphate contents.

The heavy metals detected might be from the water, soil and feeds of the livestock. The deleterious effect of heavy metal in the environment is well known (Ndiokwere and Guinn, 1983). The microbial assessment showed the presence of coliform group as well as other hazardous microbes. This by inference indicates potential presence by faecal materials and enteric pathogens. The presence of *Staphylococcus aureus* can be considered normal since this organism has been isolated from goats skin and other slaughtered animals' skin (Adegoke, 1981).

The exterior of cattle harbours large numbers of many kinds of microorganisms from soil, water, feed and manure as well as its natural

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Effects of farmyard manure and chemical fertilizers on the nutritional status of the loquat trees.

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Abstract : *The nutritional status of the loquat trees was investigated using cattle manure and commercial fertilizers for three years. The farmyard manure increased N, P, K, Mg, Fe and Zn contents of the leaves. No significant difference was found between the fertilizer types for trunk growth. Yield efficiency was nearly doubled by application of farmyard manure. Fertilizers did not affect the weight and shape of the fruits; however, commercial fertilizers led to the lower total acidity in fruits. It was concluded that the loquat trees grown in sandy soils could fulfill their principal nutrient requirements for growth and commercial yield with application of farmyard manure.*

Key words : Loquat, Plant nutrition, Farmyard manure, Commercial fertilizers.

Introduction

The loquat, being a subtropical plant, is extensively grown in the Mediterranean Basin, Japan and China, to some extent in India and Brazil with an estimated 150,000 metric tons of annual world production (Tous and Ferguson, 1996). However, there is little information on the fertilization requirement of this economically important tree fruit. The characteristic symptoms of nutrient deficiencies in loquat were described (Singh and Lal, 1990). Urea application at higher doses affected the leaf N content of young loquat trees (Singh and Shukla, 1982). A survey conducted on the loquat trees having no visible nutritional disorder revealed the strong relationship between leaf N-P-K levels and the horticultural characteristics including yield and quality (Doran 1994). With increasing leaf age, the contents of N-P-K were declined in the leaves of 15- to 18-year-old loquat trees (Ding *et al.*, 1995).

Intensive applications of N in the various forms of commercial fertilizers such as ammonium sulphate, ammonium nitrate or urea have been a widespread horticultural practice. However, concern about potential chemical contamination of ground water supplies through commercial fertilizers are encouraging environmentally safe

nutrition techniques such as farmyard manure (Maynard and Lorenz, 1979). In this work, we studied the nutritional status of the loquat trees using farmyard manure and commercial fertilizers.

Materials and Methods

This experiment was conducted for three years on the five-year-old loquat orchard (cv. Akko XIII) established at a spacing of 7×7 m at the Horticultural Research Institute / Erdemli-Icel, which is located at the Mediterranean Coastal Region of Turkey.

The amount and timing of commercial fertilizer and farmyard manure (FM) applications are given in Table-1. Increasing doses of N with tree age in the form of ammonium sulphate were applied to trees in combination with or without triple super phosphate and potassium sulphate as P and K source, respectively. Ammonium sulphate was given under the drippers of the irrigation system, while other fertilizers were placed into bands under the trees. FM was prepared through heat fermentation of cattle manure. The nutrient contents of the FM were 2.56% N, 0.69% P, 4.64% K, 12.54% Ca, 1.21% Mg, 1058 ppm Fe, 383 ppm Zn, 560 ppm Mn, and 27 ppm Cu.

Soil samples were taken with following standard procedure (Jackson, 1964) and analysed for texture, CaCO_3 , pH, soluble total salinity, organic matter, available P, Fe, Zn, Mn, Cu and exchangeable K levels (Bayrakli, 1987). The leaves were sampled from the middle of the shoots at full bloom (Doran, 1994). Total leaf N contents were determined by the Kjeldahl method and P by vanadate-molybdate yellow colorimetric method using spectrophotometer. The foliar contents of K, Ca and Mg (%), and Fe, Zn, Mn and Cu (ppm) were assessed by Atomic Absorption Spectrophotometer after ashing at 550°C and extraction in 10% hydrochloric acid (Chapman *et al.*, 1961; Bayrakli, 1987).

Trunk circumferences were measured 10 cm above the bud union of the trees in January when the vegetative growth was minimum (Perez, 1983). Cumulative yields were obtained from all fertilizer applications. The weight, width and length of fruits, number of seeds, soluble solids, acidity and pH were determined on 25 marketable fruits in the replications (four trees each replication) in each randomized blocks.

The statistical analyses were conducted using a computer program, TARIST (Açıkgöz *et al.*, 1993). The means (16 combinations for commercial fertilizers and 5 for FM) were compared by Duncan test.

Results and Discussion

The experimental soil was loamy sand texture without salinity. It had slightly high pH with high lime and low organic matter content. Total N and available K, Fe, Zn contents were at insufficient levels, while available P, Ca, Mg, Mn, Cu and B contents were sufficient (Table-2). The experimental soil can be considered suitable for loquat culture (Rajput and Teskey, 1979; Perez, 1983; Doran, 1994).

The optimum leaf nutrient levels of Akko XIII loquat variety (Doran, 1994) and mean values of analysis of leaf samples taken from each fertilizer application were given in Table-3. The applications of ammonium sulphate, triple super

phosphate and potassium sulphate individually affected foliar N, P and K contents, respectively. This positive response of loquat trees to the major commercial fertilizers has long been confirmed in other fruit trees (Anonymous, 1992). However, foliar N, P and K contents were found to be higher in trees received farmyard manure in comparison to those received the chemical fertilizers. The foliar N contents ranged from 1.55% to 1.61% in trees that received FM. Similarly, in the higher doses of FM applications, P and K contents of leaves were found as high as 0.13%. This indicated that FM could be a suitable N, P, and K source to the loquat trees (Doran, 1994).

For foliar, Ca or Mg contents, the difference between trees with and without potassium sulphate applications was statistically significant. This was probably due to the antagonistic effects of K ions in the soil solution on Ca and Mg ions (Kacar, 1983; Ozbek *et al.*, 1984; Mengel *et al.*, 1987). FM applications resulted in higher foliar Mg content. Mg is an important component of chlorophyll molecule and its deficiency causes of poor chlorophyll content of leaves, leading to the reduction of photosynthetic rate (Faust, 1989).

FM applications also increased foliar Fe and Zn contents. The foliar Fe content was as high as 116.3 ppm in the highest farmyard manure dose. Similarly, relatively increased foliar Zn content (over 22 ppm) was found in FM application. It was reported that Zn ions had a positive effect on the nitrogen metabolism of plants (Kacar, 1983), which in turn might affect the vegetative growth. There were no statistically significant differences between the doses or fertilizer types in respect to foliar Mn or Cu contents. This may be due to their sufficient levels in the experimental soil.

Effects of commercial fertilizer combinations and FM on the growth, yield and some fruit quality characteristics of loquat cv. Akko XII are given in Table-4. FM applications caused in a similar affect as commercial fertilizer combinations did on the growth of trunk cross

sectional areas. These findings might confirm that N requirement of loquat trees for vegetative growth can be sufficiently replaced by the use of FM applications. This is especially important for trees

grown in sandy soils in which FM applications are expected to improve the uptake of N (Rajput and Teskey, 1979; Perez, 1983; Ozbek *et al.*, 1984; Anonymous, 1992; Doran, 1994).

Table - 1 : The amount and timing of commercial and farmyard manure applications to the loquat trees.

Fertilizers	Doses	Given amount of the fertilizers			Timing
		1993	1994	1995	
Ammonium sulfate (g N/tree)	I N	0	0	0	40% post harvest (June)
	II N	225	340	510	40% before cluster burst (August)
	III N	450	68	1020	20% at the beginning of fruit development (March)
	IV N	675	1020	1530	
Triple super phosphate (g P ₂ O ₅ /tree)	I P ₂ O ₅	0	0	0	All applied before cluster burst (August)
	II P ₂ O ₅	180	270	410	
Potassium sulfate (g K ₂ O/tree)	I K ₂ O	0	0	0	50% before cluster burst (August)
	II K ₂ O	270	410	600	50% at the beginning of fruit development (March)
	I	18	27	40	
Farmyard manure (FM) (kg/tree)	II	36	54	80	
	III	54	81	120	All applied before cluster burst (August)
	IV	72	108	160	
	V	90	135	200	

Table - 2 : Some physical and chemical properties of the orchard soil.

Soil depth (cm)	Texture	Total soluble salt%	pH	Lime%	Organic matter%	Total N%	Available nutrients (ppm)								
							P	K	Ca	Mg	Fe	Zn	Mn	Cu	B
0-20	SL*	0,014	7,7	21,4	1,39	0,06	11	135	10853	514	2,7	1,0	4,2	0,5	2,3
20-40	LS**	0,011	7,8	20,3	1,85	0,08	7	109	11571	638	3,6	1,1	3,7	0,3	1,6
40-60	LS**	0,011	7,8	23,7	1,03	0,06	5	86	11247	703	3,9	0,4	2,9	0,3	1,3
60-90	S***	0,010	8,1	15,8	0,61	Min.	2	52	10117	496	2,1	0,3	2,1	0,2	0,7

* Sandy-Loamy

** Loamy-Sandy

*** Sandy

The yield (kg per tree) or yield efficiency (kg per cm² of cross sectional area of trunk) were higher in trees grown on FM. The yield efficiency, which is often considered the most important criteria in comparing the productivity of fruit trees, was nearly doubled by the use of maximum FM dose (0.532 kg/cm² by FM vs 0.287 kg/cm² by commercial). The type of fertilizers did not affect the weight and shape of fruits. Soluble solids and pH were increased by the use of FM. However, commercial fertilizers gave the relatively lower total acidity in loquat fruits. This suggested that higher or excess doses of organic fertilizers might decrease the taste of loquat fruits. Detailed studies rather than system comparison are needed to reveal the qualitative difference between organic and conventional cultivation (Woese *et al.*, 1997).

There are some limitations for the use of FM, since they are variable in decompositions and nutrient release and high in cost per unit plant nutrient supplied (Maynard and Lorenz, 1979). But, in the case of commercial fertilization, 90% nitrogen from ammonium sulphate nitrified and leached from the fertilizer band within 30 days after application to a fine sandy soil (Lorenz *et al.*, 1972). The application of organic fertilizers satisfied the nutrition demand of the olive trees and increased the yield drastically compared with conventional nutrition (Stravroulakis, 2000). This confirmed the replacement potential of chemicals with organic fertilizers as seen in our study. Furthermore, FM secures the nutrients available continuously to the trees (Kaburakis, 1999).

From this work, it can be concluded that loquat trees may fulfill their major nutrient requirements for growth and commercial yield with

FM applications in sandy type soils. The increasing price of commercial fertilizers and concerns about their pollutant characteristics are

Table – 3 : The effect of fertilizer types and doses on the leaf nutrient contents of loquat trees.

Nutrients	Fertilizers and doses												
	Ammonium sulfate				Triple super phosphate		Potassium sulfate		Farmyard manure (FM)				
	I	II	III	IV	I	II	I	II	I	II	III	IV	V
N 1,35-1,66 ^y Duncan	1,47** b ^z	1,52 a	1,54 a	1,56 a	1,50* b	1,54 a	1,52	1,52	1,55	1,59	1,61	1,60	1,60
P 0,106-0,118	0,11	0,10	0,10	0,11	0,10** b	0,12 a	0,11	0,12	0,11	0,12	0,12	0,13	0,13
% K 1,03-1,39	1,10	1,09	1,07	1,13	1,07* b	1,13 a	1,04** b	1,15 a	1,17* b	1,24 ab	1,23 ab	1,30 a	1,31 a
Ca 2,10-2,94	2,95	2,85	3,14	2,98	2,93	3,03	3,13** a	2,83 b	3,03** a	2,97 ab	2,60 bc	2,60 bc	2,30 c
Mg 0,33-0,39	0,38	0,38	0,39	0,39	0,38	0,39	0,41** a	0,36 b	0,37	0,42	0,42	0,42	0,43
Fe 73,6-84,6	95,50	95,83	91,83	89,00	95,50	90,58	91,75	94,33	85,00* c	97,00 bc	101,7 abc	102,9 ab	116,3 a
Zn 18,2-23,1	19,2** b	20,11 ab	19,08 b	21,21 a	19,64	20,15	19,82	19,97	23,17	22,30	22,57	23,97	23,17
Mn 21,5-26,0	21,80	23,48	23,42	23,66	23,31	22,87	23,60	22,58	24,60	28,63	26,73	27,80	28,83
Cu 3,1-11,3	103,9	91,25	82,42	89,67	94,46	89,17	88,75	94,87	81,67	93,00	107,3	92,33	80,00

* Significant at the % 5 level of probability;

** Significant at the % 1 level of probability

^z Mean separation significant within column by Duncan's multiple range test

^y Optimum levels of standard values (Doran, 1994).

Table – 4 : The effect of fertilizer types on the growth, yield and some fruit quality characteristics.

Fertilizer type	Dose	Characteristics								pH*
		Trunk crosssectional area** (cm ²)	Yield** (kg/tree)	Yield** efficiency (kg/cm ²)	Fruit weight (g)	Index width / length	Ratio of seed* (%)	Soluble solids** (%)	Total acidity (%)	
Commercial fertilizers with combinations of N-P-K	Minimum	31,64	8,82	0,287	30,33	0,82	15,64	9,20	0,67	4,1
	Average	33,02	12,10	0,372	32,43	0,85	16,67	9,75	0,74	4,2
	Maximum	38,69	16,10	0,473	35,93	0,86	18,28	10,27	0,81	4,3
Farmyard manure	Minimum	29,21	11,43	0,399	32,37	0,82	14,87	9,97	0,71	4,3
	Average	32,93	15,55	0,477	33,88	0,84	15,55	10,33	0,74	4,3
	Maximum	37,02	19,07	0,532	35,63	0,86	16,34	10,63	0,78	4,3

* significant at the %5 level of probability

** significant at the %1 level of probability

expected to reduce their use in the coming years. In addition, the recent trend for the organic farming techniques may encourage them to minimize the use of chemical fertilizers in their orchards. With more understanding of the benefit of organic fertilization, orchardists will be eager to use environmentally safe sources for satisfactory production, while not disturbing the environment.

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Brainstem auditory evoked responses in young urban and rural boys - A comparison.

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Abstract : Brainstem auditory evoked responses (BAER) were studied in a total of 194 boys in the age group between 10-15 yrs taken from a busy metropolitan city and also from a relatively quiet town in order to compare their values and to look into the probable cause of the difference between them, if any. BAER were tested with the help of Compact-4 (Nicolet, USA) instrument using the standard technique. In general, the BAER values of the two sample populations were found to be almost similar with no gross differences in terms of peak latency and inter peak latencies. The values were found to be comparable to adult male values as reported earlier.

Key words : BAER peak latencies, Inter peak latency.

Introduction

Ambient noise is a frequent, unavoidable and continuously increasing environmental factor of modern life. Welch and Welch (1970) while analyzing the physiological effects of noise on the human emphasised that environmental sound influences virtually every organ system and function. Noise acts as an environmental and occupational stressor on the human organism. Extra-auditory effects of noise are defined as those effects on health and well being, other than hearing system, that are caused by exposure to noise. Studies in adult males have investigated the influence of road traffic noise on circulatory variables. The results indicated either moderate increase in systolic blood pressure and diastolic blood pressure in males exposed to noise (Babisch *et al.*, 1988) or no difference in systolic and diastolic blood pressure or in heart rate (Babisch *et al.*, 1993). Although a great deal of work in the field of extra-auditory effects of noise on the human has been carried out, the effects of noise on the central nervous system have not been studied in detail. Brainstem Auditory Evoked Response (BAER) has become a useful tool in assessing the functional integrity of the conduction pathway of the sound wave from the receptor to the cerebral cortex. Though many workers have reported

normative data of the BAER in their subjects (Hecox and Galambos, 1974; Rowe, 1978; O'Donovan *et al.*, 1980; Mochijuki *et al.*, 1983 and Thivierge and Cote, 1987), the data are rather scarce in the children. Data are also lacking in comparing BAER of normal populations taken from different habitats in terms of day-to-day ambient background noise level. Noise pollution has already caused a concern for the health, both physical and mental, of the people in general, particularly in the growing children. The hypothesis of the present study is that exposure of growing children in urban environment with higher ambient noise level may affect the auditory mechanism. Hence, in the present study, an attempt has been made to assess BAER of normal healthy young boys in the age group of 10-15 years taken from a busy metropolitan city in order to assess the effect of ambient noise on the auditory function and to compare with those of a quiet suburb.

Materials and Methods

A total of 194 boys in the age between 10 - 15 years, out of which 100 from a school located in a metropolitan city (referred in the text as urban) and another 94 boys from a different school located at a remote place away from urban zone

(referred in the text as rural), selected at random, were taken as the subjects of the present study. They had normal hearing as assessed by audiometry. The physical characteristics of the subjects are given in Table 1. Subjects were briefed about the experimental procedures and informed consent was obtained. The average ambient noise level as monitored during the school hours were 84.48 ± 2.35 dBA and 65.62 ± 4.73 dBA in urban and rural areas respectively. The BAER of the right and left ear were recorded using the Nicolet Compact - 4 instrument (Nicolet, USA). BAER recordings were carried out in the morning hours after a light breakfast, in a dimly lit laboratory under controlled environmental conditions.

Electrodes were attached at the vertex (Cz) and the ear lobes, with ipsilateral ear lobe serving as the earth. Monaural auditory stimuli consisting of clicks of 100 μ sec square pulses were delivered through an electrically shielded earphone at the rate of 15/sec. The intensity was 70 dB above the click-hearing threshold.

Table -1 : physical characteristics of the subjects.

Age (years)	10		11		12		13		14		15	
	wt (kg)	ht (cms)	wt (kg)	ht (cms)	wt (kg)	ht (cms)	wt (kg)	ht (cms)	wt (kg)	ht (cms)	wt (kg)	ht (cms)
Urban	24.2	129.3	28.4	136.7	30.9	143.8	34.6	147.7	39.8	155.0	42.7	160.0
Boys	± 2.81	± 5.94	± 3.17	± 5.22	± 3.96	± 5.38	± 6.02	± 9.45	± 7.59	± 6.04	± 7.30	± 7.63
N	14		13		16		14		15		13	
Rural	27.3	134.4	28.9	136.3	28.8	136.2	34.7	145.2	39.2	151.7	43.8	159.6
Boys	± 4.30	± 8.17	± 4.08	± 7.75	± 4.63	± 7.66	± 4.65	± 5.53	± 7.85	± 10.82	± 5.23	± 5.77
N	16		21		18		20		15		19	

Values are Mean \pm SD

N = Number of subjects

V (Table-3), there was not much difference between urban and rural boys.

The study was conducted to compare the BAER data of the children in the metropolitan area and a remote, quiet area. In general, the BAER values in all the age groups were reasonably close. These data were comparable to those reported on children as well as adult male Indian population

The evoked electrical activity was amplified 10,000 times and a band pass of 150 - 3000 Hz was used to filter out the low and high frequency electrical noise. It was averaged over 2000 click presentations for 10 msec sweep time. The averaged evoked response was displayed and printed on a paper. At least two trials were obtained from each side of stimulation to ensure reproducibility of the responses. The peak latency of Wave I, III and V and inter peak latency of I-III, III-V and I-V were analysed.

Statistical Analysis : Unpaired Student's 't' test was used to compare the BAER in the two groups.

Results

The mean \pm SD values of the latencies of the different peaks of BAER from each ear in the two samples of the subjects of the different ages are given in Table 2. The peak latencies of I, III and V of both the ears of the subjects were found to be similar in both the groups. Similarly for the interpeak latencies (IPL) between I-III, III-V and I-

(Tandon, 1990; Mukhopadhyay *et al.*, 1992; Tandon and Krishna, 1990). Since the measurement of BAER clinically reflects the conduction pathway of sound from the auditory receptor to the cerebral cortex, the similarity in responses of the two groups of age-matched boys indicated that the two did not differ in respect of the concerned neurosensory mechanisms. The abnormalities in BAER were classified as sensory,

Auditory evoked potential in children.

neural or sensory-neural loss based on the following criteria. If the peak latency of wave I shows delay, with a normal peak latency and IPL

of other waves, the condition has been designated as 'sensory' or cochlear involvement. On the other hand, when the peak latency of wave I is normal

Table -2 : Auditory brainstem responses of both the ears from the subjects in different age groups.

Age (years)		Right ear			Left ear		
		Peak latencies (msec)			Peak latencies (msec)		
		I	III	V	I	III	V
Urban boys	10	1.76 ±0.18	3.79 ±0.14	5.66 ±0.15	1.76 ±0.16	3.77 ±0.15	5.61 ±0.16
	11	1.78 ±0.15	3.84 ±0.25	5.68 ±0.24	1.76 ±0.12	3.97 ±0.18	5.68 ±0.26
	12	1.73 ±0.10	3.87 ±0.25	5.76 ±0.29	1.69 ±0.13	3.77 ±0.28	5.76 ±0.25
	13	1.73 ±0.13	3.88 ±0.22	5.67 ±0.21	1.73 ±0.30	3.85 ±0.20	5.56 ±0.18
	14	1.79 ±0.14	3.83 ±0.19	5.71 ±0.34	1.81 ±0.25	3.94 ±0.27	5.69 ±0.26
	15	1.74 ±0.11	3.77 ±0.14	5.53 ±0.23	1.75 ±0.19	3.88 ±0.35	5.61 ±0.36
	10	1.76 ±0.16	3.89 ±0.29	5.81 ±0.36	1.74 ±0.16	3.88 ±0.26	5.81 ±0.29
	11	1.79 ±0.10	3.89 ±0.29	5.78 ±0.24	1.77 ±0.18	3.93 ±0.28	5.76 ±0.30
	12	1.79 ±0.17	3.81 ±0.19	5.72 ±0.23	1.79 ±0.23	3.84 ±0.19	5.74 ±0.30
	13	1.85 ±0.19	3.88 ±0.21	5.68 ±0.22	1.81 ±0.17	3.93 ±0.24	5.69 ±0.28
Rural boys	14	1.85 ±0.19	3.91 ±0.20	5.77 ±0.24	1.83 ±0.17	3.91 ±0.17	5.79 ±0.23
	15	1.86 ±0.18	3.96 ±0.23	5.87 ±0.47	1.85 ±0.14	3.98 ±0.25	5.84 ±0.27

Mean ± SD

and IPL of I-III and III-V are prolonged, the condition is termed as neural or retro-cochlear. If the delay occurs in both peak latency of wave I as well as in IPL of I-V, then it is termed as sensory-neural or involving both cochlear and retro-cochlear pathways. Though the two groups belonged to the two areas of varying ambient noise environments, no gross changes were seen in BAER. Probably the difference in the level of noise

in the urban (with 85 dBA) was not sufficient to cause any obvious change in the integrity of the auditory pathway. From the observations of the present study, when the two age and sex matched samples of boys population, differing in physical (urban noise level) attributes, appear to have similar BAER values that are comparable to adult values reported elsewhere, it is prudent to conclude that brainstem evoked potential are remarkably

stable within the reasonable variation in physical and physiological environments. It is thus suggested that the presently reported BAER data

on the boys may be considered as the normative values for Indian young boys population.

Table -3 : Interpeak latencies of auditory brainstem responses of both the ears from the subjects in different age groups.

Age (years)		Right ear			Left ear		
		Interpeak latencies (msec)			Interpeak latencies (msec)		
		I –III	III-V	I-V	I –III	III-V	I-V
Urban boys	10	2.04 ±0.12	1.86 ±0.12	3.90 ±0.16	2.01 ±0.10	1.84 ±0.17	3.85 ±0.13
	11	2.07 ±0.25	1.84 ±0.19	3.90 ±0.20	2.01 ±0.19	1.89 ±0.16	3.89 ±0.22
	12	2.14 ±0.21	1.89 ±0.17	4.04 ±0.27	2.09 ±0.23	1.99 ±0.21	4.08 ±0.24
	13	2.15 ±0.18	1.79 ±0.14	3.94 ±0.15	2.13 ±0.32	1.71 ±0.26	3.84 ±0.37
	14	2.03 ±0.20	1.88 ±0.33	3.99 ±0.24	2.12 ±0.15	1.74 ±0.26	3.94 ±0.20
	15	2.04 ±0.12	1.76 ±0.19	3.80 ±0.21	2.13 ±0.23	1.73 ±0.23	3.86 ±0.30
	10	2.13 ±0.23	1.92 ±0.26	4.05 ±0.28	2.15 ±0.24	1.92 ±0.29	4.07 ±0.25
	11	2.11 ±0.26	1.89 ±0.27	3.96 ±0.28	2.16 ±0.22	1.83 ±0.21	3.99 ±0.31
	12	2.02 ±0.25	1.90 ±0.27	3.92 ±0.23	2.04 ±0.21	1.90 ±0.20	3.95 ±0.21
	13	2.03 ±0.18	1.89 ±0.14	3.83 ±0.19	2.12 ±0.18	1.77 ±0.22	3.89 ±0.25
	14	2.06 ±0.14	1.86 ±0.14	3.92 ±0.23	2.07 ±0.19	1.88 ±0.22	3.96 ±0.20
	15	2.09 ±0.17	1.87 ±0.25	4.00 ±0.40	2.13 ±0.19	1.85 ±0.15	3.98 ±0.25

Mean ± SD.

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Auditory evoked potential in children.

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Effect of the extract of *Thespesia populnea* leaves on mice testis.

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Abstract : Male swiss mice were administered with the leaf extract of *Thespesia populnea* at a daily dose of 400 mg/kg body weight for 15 days and the testis were subjected to structural analysis. The structure of the seminiferous tubule in the testis of treated animal was elongated. Sertoli cells were enlarged in its structure and spermatids became round and disintegrated. It is suggested that the extract of *T. populnea* treatment leads to pathological changes in the seminiferous tubules, sertoli cells and spermatids of the testis.

Key words : Extract, Testis, Seminiferous tubule, Spermatids, Sertoli cells.

Introduction

Hundreds of plants are identified to cause disturbance in spermatogenesis and numerous plant extracts have been tested for this purpose. To mention a few alcoholic extract of *Hibiscus rosasinensis* causes antifertility effect in male albino mice (Madhusudana Reddy *et al.*, 1997); *Azadirachta indica* extract arrests spermatogenesis (Ravindaranath *et al.*, 1999); alkaloids of *Vinca rosea* depletes the sperms in seminiferous tubules (Stanley and Akbarsha, 1992). Laboratory experiments on rat and mouse models have shown that VCR (Vincristine) arrests both mitosis and meiosis in the spermatogenic compartments of the testis, impairing the spermatogenesis (Lee and Dixon, 1972; Parvinen *et al.*, 1978; Moore *et al.*, 1986). Plant extract of *Mollugo stricta* induces spermatostatic and antifertility effects in male and female mice (Padma *et al.*, 1992). The present study was undertaken to understand the effect of acetone extract of leaves of *Thespesia populnea* (Family : *Malvaceae*) on the testes of swiss albino male mice.

Materials and Methods

The leaves of *T. populnea* were collected locally, cleaned and dried in shade, powdered and subject to Soxhlet extraction successively with acetone. The extracts were concentrated under reduced pressure and controlled temperature (50-

60°C). Experimental doses of the extract were prepared by dissolving it in refined coconut oil (1gram / 10 ml). Male albino mice of swiss strain 80-90 days of age, were purchased from JIPMER, Pondicherry, and fed with standard pellet (Gold Mohur Feeds, Lipton India Ltd., Bangalore, India) and water *ad libitum*. The mice were divided into two groups of six animals each. The first group served as control and was administered with 0.1ml of coconut oil (carrier) for 15 days and the second group was given orally 400mg / kg body weight of leaf extract for 15 days. Subsequently the mice were killed by decapitation and dissected to remove the testis. The testes were fixed in Bovin-Hollande fixative, used to obtain paraffin sections (4-6µ) and stained with Ehrlich haematoxylin and eosin. Photomicrographs were obtained using Nickon Light microscope (Japan).

Results

The leaves of *T. populnea* extract treated male mice revealed pathological changes in the testis. Seminiferous tubules of the testis of treated mice were reduced in size and elongated in shape. The Leydig cells were dense and compact in the control but necrotic and highly vacuolated in the treated mice. The sertoli cells were spaced at regular intervals and had indistinct outlines with oval shape in the control animal's testis. These cells were enlarged and their nuclei were intact in the treated mice. Small spherical structures of

spermatids had a close association with sertoli cells in the seminiferous tubules of the control mice. In the treated mice, spermatids were distinct

with prominent nuclei, reduced in number, and shrunk in size. The lumen of the seminiferous tubule got disintegrated and appeared vacuolated

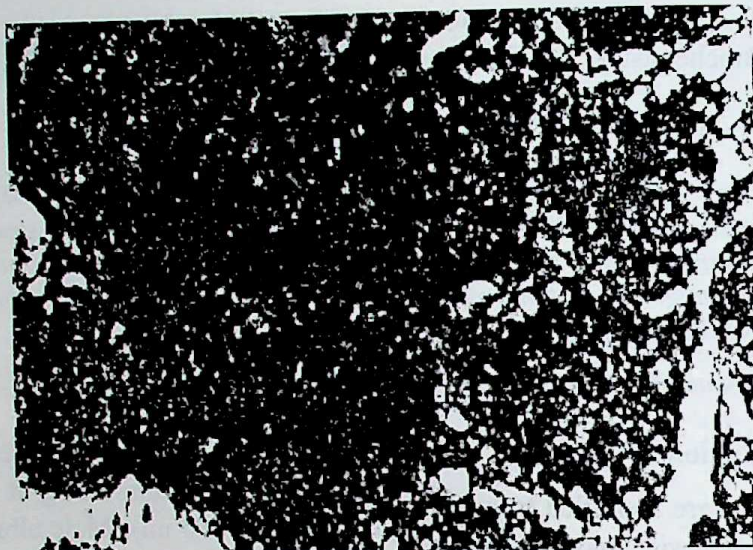


Fig. 1 : Seminiferous tubules of a control mice.



Fig. 2 : Magnified single tubule shows different types of cells (St-spermatids, PSC-Primary spermatocyte, SC-sertoli cells, LC-Leydig cells).

and the number of primary secretory cells increased in the treated animals.

Discussion

The treatment of acetone extract of *T. populnea* leaves on male mice has shown histopathological changes in the seminiferous tubules, Leydig cells, Sertoli cells and Spermatids of testis. The Leydig cells are scattered and

disappear, within the interstitial space, which may reduce the reproductive ability. In the vincristine treated male rats, the nuclei as well as cytoplasm of Leydig cells were affected and decreased the levels of circulating androgens (Stanley and Akbarsha, 1994). The changes observed in the Leydig cells reflect cellular necrosis (Wyllie, 1981). As the Leydig cells in the various extract of *H. rosasinensis* treated testis indicates the

Effect of the extract of Thespesia populnea on mice.

inefficiency of Leydig cells to synthesis testosterone (Madhusudana Reddy *et al.*, 1997). Pathological changes observed in the sertoli cells and appearance of large and intact nuclei reflect

cellular changes in the seminiferous tubule. Sertoli cells and spermatogenic elements depend on androgen support, the pathological changes in the Leydig cells were reflected as disturbances in



Fig. 3 : Elongated structure of seminiferous tubules of *Thespesia populnea* extract treated (400mg/Kg body weight) 15 days old mice.

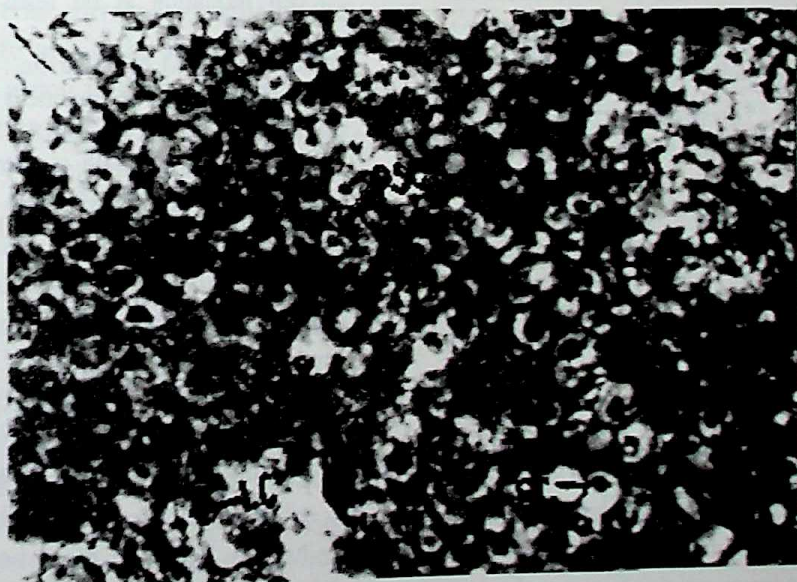


Fig. 4 : A part of Fig. 3. magnifies showing enlarged and disintegrated cells of the 15 days. *T. populnea* extract treated mice.

spermatogenesis (Skinner, 1991). The reduction in the number of spermatids may be due to the non-availability of gonadotrophins. The non-availability of FSH could be because of inhibition of gonadotrophin release from the pituitary (Clermont and Hermo, 1985). The *Azadirachta*

indica extract causes varying degree of arrest of spermatogenesis and degeneration of the nuclei germinal cells in male mice (Ravindaranath *et al.*, 1999). *T. populnea* extract has been reported to possess antifertility in albino female mice (Kavimani *et al.*, 1999). Microtubules of sertoli

cells cannot escape from the damage by extract of *Vinca rosea* (Akbarsha *et al.*, 1996). Decreased diameter in size and elongated in its structure of the seminiferous tubule in treated with *T. populnea* extract. Similarly, changes in the seminiferous tubule are proportion to *Hibiscus rosasinensis* treated albino male mice (Madhusudana Reddy *et al.*, 1997). The structural changes in the seminiferous tubule were lead to antispermatogenic activity.

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Heavy metal pollution in various canals originating from river Yamuna in Haryana.

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Abstract : Heavy metal pollution due to Fe, Ni, Pb, Cd, Co and Zn in the water of major canals originating from the river Yamuna in Haryana was studied. All these metals except Zn were found to be present in the Western Yamuna Canal (WYC) exceeding the maximum permissible limits. In the Sunder branch (SB), the heavy metal concentration was relatively more. Concentrations of the metals were, however, relatively less in the highly eutrophicated waters of Agra canal and Gurgaon canal as compared to that in WYC but Fe concentration were much higher. Except Zn and Ni the metal concentrations exceeded the standard permissible limits in these canals also.

Key words : Heavy metals, Pollution, Canals, Industrial effluents.

Introduction

Haryana is mainly an agriculture-based state, but in the recent years, it has shown fast industrial development as well. The industrial growth of Haryana is concentrated mainly in the eastern parts of the state along the river Yamuna, the major river flowing along the eastern border of the state from which a number of important canals branch off. These canals are mainly responsible for supplying water to the majority of the districts of the state for irrigation and drinking. Huge quantities of industrial and urban wastes and agricultural run-off find their way directly or indirectly into the river Yamuna and its canals and several heavy metals present therein pollute the water and move through the aquatic food chain. When used for irrigation, the canal water containing heavy metals can have serious toxic effects on the growth and yield of crops and when used for drinking it can lead to serious health disorders (Underwood, 1971).

It is therefore, very important to study the concentration of heavy metals in water bodies and in the recent past there has been increasing emphasis on studies related to trace metal pollution of various rivers (Ranu *et al.*, 1991; Sharma *et al.*, 1993; Prebha and Selvapathy, 1997; Kaushik *et*

al., 2000 and 2001). The present study on water quality of major canals of Haryana was undertaken since there have been no systematic studies on the same in the state. In the present study the concentration of heavy metals viz. Fe, Ni, Pb, Cd, Co and Zn were determined at various stations all along the route of the major canals originating from the river Yamuna in Haryana with a view to assess their suitability for safe drinking and/or irrigation in the state. These canals included Western Yamuna Canal (WYC) and its Sunder branch (SB), Agra Canal (AC) and Gurgaon Canal (GC). The metal concentrations in these canals were corroborated with potential sources of pollution at various sites.

Description of study sites : The Western Yamuna Canal originates from the river Yamuna at Tajewala barrage near Hathinikund in Haryana. The water of WYC is used mainly for irrigation and also for drinking purposes. The WYC flows through the industrial belts of Yamuna nagar, Karnal, Panipat and Sonapat just parallel to the river Yamuna before entering Delhi.

The WYC branches off at Munak head into two branches i.e., SB and WYC main. Sunder Branch is mainly used for irrigating the Hansi and Tosham areas of Hisar and Bhiwani whereas the

WYC main reaches Delhi at Haiderpur Water Works, where its water is treated and supplied for drinking purposes to the people of West Delhi.

The Agra canal emerges from River Yamuna at Okhla Barrage in Delhi and passes through Faridabad, Ballabhgarh, Palwal and Hodal

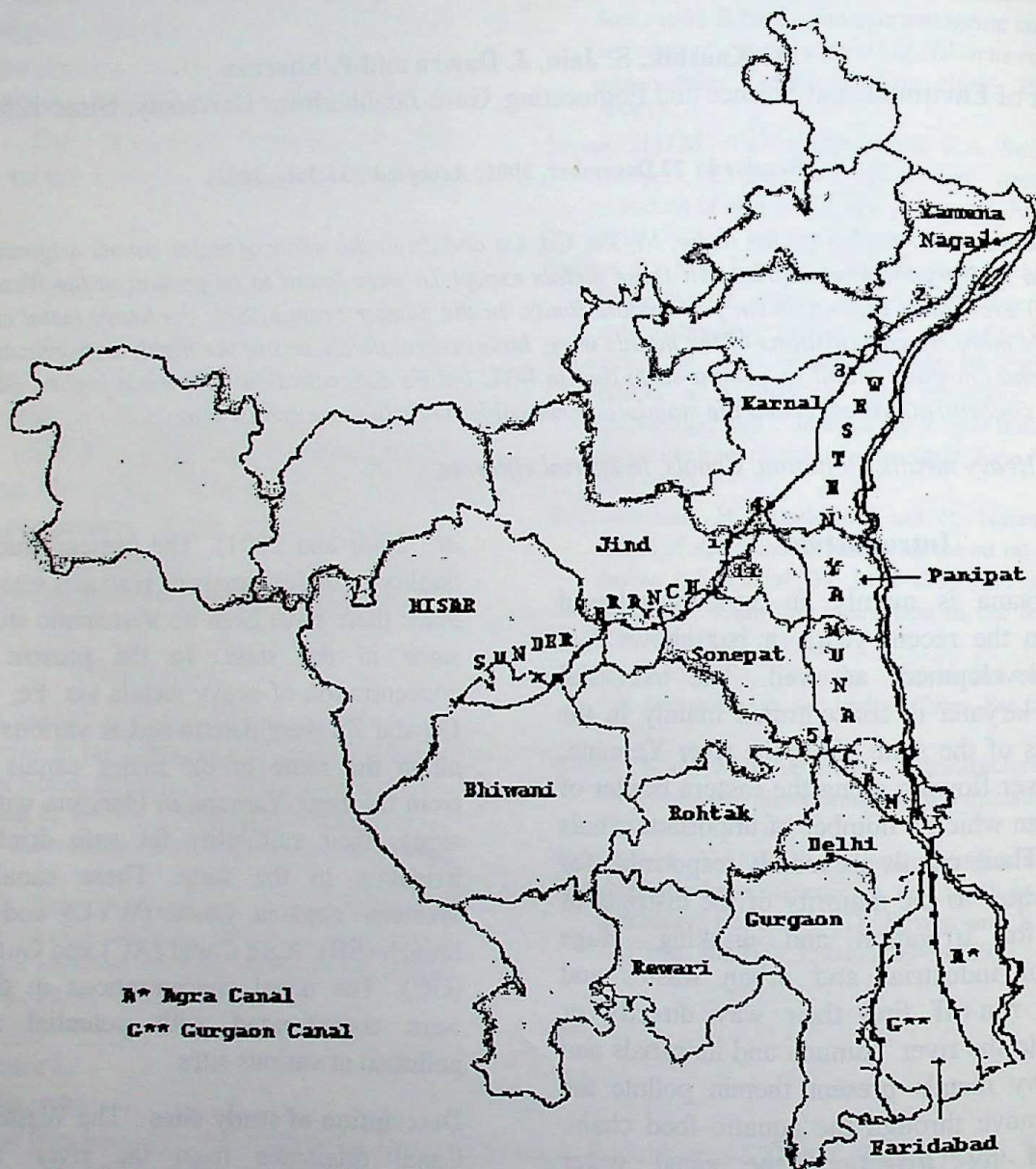


Fig. 1 : Sampling stations of western Yamuna canal, Sunder branch, Agra canal, Gurgaon canal in Haryana.

Western Yamuna canal	Sunder branch	Agra canal	Gurgaon canal
1. Tajewala head	i. Safido head	a. Okhla	A. Meethapur
2. Yamuna nagar	ii. Bada urlana	b. Fbd. 37 bdge.	B. Mowai
3. Karnal	iii. Saifabad	c. Old fbd. bdge.	C. Banoli
4. Munak	iv. Mall savanna head	d. Chandwali bdge.	D. Pratapgarh
5. Haiderpur treatment plant	v. Bodipul	e. Mandkola bdge.	E. Bijopur
	vi. Gatauli	f. Janoli	F. Mindkola
	vii. 100 m away from Gatauli	g. Ghodi	G. Ujjina
	viii. Karela	h. Bhiduki	
	ix. Baas		
	x. NH-bridge (Mundhal)		

regions of Southern Haryana before entering into U.P. The Gurgaon canal bifurcates from Agra canal at Meethapur in Haryana and flows parallel to Agra canal. It passes through Faridabad, Gurgaon, Sohna, and Hodal and leaves Haryana at Punhana.

Sampling of water was done from major stations all along the route of various canals. Site specifications for the canals are shown in Table 1 and positions of the canals are depicted in the map (Fig 1).

Materials and Methods

Water sampling and analysis : Sampling of WYC water from Tajewala barrage to Haiderpur treatment plant, SB water from Safido head to NH-10 bridge, Agra canal water from Okhla to Hasanpur and Gurgaon canal water from Meethapur to Ujjina was done in February, 1999. Grab samples in triplicate were taken from each station and the water was collected in high-grade polyethylene bottles of 2L capacity. The samples were stored in iceboxes until brought to the laboratory.

The water samples were filtered through Whatman Filter Paper to remove any suspended particles and acid digested using HNO_3 for minimizing the interference by organic matter prior to the estimation of heavy metals by double beam atomic absorption spectrophotometer (Varian Spector AA 20 plus) using acetylene gas as the fuel (at 8 psi) and air and nitrous oxide as supporting gases. AR-grade purified metals and metal oxides were used for preparing various standards for calibration following Standard Methods (Clesceri *et al.*, 1996).

Results and Discussion

Concentrations of various heavy metals in WYC (main and Sunder branch), Agra canal and Gurgaon canal are depicted in Table 2.

Western Yamuna canal : Water of Western Yamuna canal is mainly used for irrigation and drinking purposes. Various domestic and industrial

effluents are carried to the WYC from Yamuna nagar, Karnal, Panipat and Sonipat.

The concentration of Fe increased many folds in WYC on its way from Tajewala Head (0.14 mg/l) to Haiderpur treatment plant (2.04 mg/l) as it flowed downstream to the major industrial cities. Maximum nickel concentration in the WYC was at Yamuna nagar downstream, which is an industrial town of Haryana but decreased after that (Table 2). A similar trend was also observed for Pb, Co and Zn. However, Cd concentration remained homogenous throughout the canal.

Sunder Branch (SB) : This branch of WYC flows through rural areas mainly where agricultural activity predominates. Fe concentration increased from 0.29 to 0.74 mg/l as the canal flowed from station SB-01 to SB-04. Thereafter, the concentrations tended to decrease.

Ni and Pb in the SB were higher as compared to that in WYC. Concentration of Cd throughout the branch was uniformly in the range of 0.02-0.03 mg/l. Co and Zn concentrations were particularly high in the Sunder Branch. In SB waters, all the heavy metals barring zinc were present in concentrations more than the permissible levels, which can seriously affect the crop yield when used for irrigation.

The Western Yamuna Canal carries a considerable amount of water throughout the year, whereas in SB, the water level was quite low and the subsequent high rate of evaporation may be leading to increased metal concentration. Relatively higher concentration of most of the heavy metals at WYC-02 and WYC-03 may be attributed to inputs received from the industries and the drains carrying the municipal wastes from Yamuna nagar and Karnal. On the other hand, the presence of heavy metals at WYC-01 where there is hardly any industrial activity could be due to agricultural inputs or may be from the Yamuna river itself due to geological origin. The authors also reported high metal concentrations in the river Yamuna even at its point of origin at Hathinikund

(Kaushik *et al.*, 2001). The WYC, which comes out at Tajewala barrage near Hathinikund, has almost similar or lesser concentrations of the heavy metals with the only exception of lead. A sharp increase in Fe concentration at Haiderpur (2.04mg/l) can be related to the discharge of effluents from industrial area of Gohana-Sonepat complex through drain no.2 and 8.

Concentration of Fe in the water of WYC at 60% of the stations, Ni in 40% and Pb, Cd and Co at all the stations are found to cross the permissible limits. Iron, though an essential element is discarded beyond 1 ppm due to bitter taste. It also causes respiratory problems in fishes by covering their gills as iron hydroxides (Oladimeji and Offeon, 1989). It is also unsuitable

Table - 1 : Sampling Locations of Western Yamuna Canal (WYC), Sunder Branch (SB), Agra Canal (AC) and Gurgaon Canal (GC) in Haryana.

Sampling station	Distance from origin (kms)	Land use in adjoining area
Western Yamuna canal		
WYC-01 Tajewala head	2	Rocky, agricultural, residential area
WYC-01 Yamunanagar	40	Industrial area; Yamunanagar
WYC-03 Karnal	125	Complex (distillery, sugar, starch utensils, paper mill)
WYC-04 Munak		Agricultural zone
WYC-05 Haiderpur treatment plant	236	-do-
Sunder branch		
SB-01 Safido head	0	Industrial zone Haiderpur water works
SB-02 Bada Urlana	21	Agricultural area
SB-03 Saifabad	35	-do-
SB-04 Mall Savana head	46	-do-
SB-05 Bodipul (Nandgarh) RD 33	56	-do-
SB-06 Gatauli	67	-do-
SB-07- 100 m away from Gatauli	67	-do-
SB-08 Karela	78	Agricultural area (mixing of ground water)
SB-09 Bass	87	Agricultural area
SB-10 National highway bridge, Mundhal	100	-do-
Agra canal		
AC-01 Okhla head	0	Abundant foaming, eutrophication in standing water
AC-02 Faridabad 37 bridge	6	Industrial and residential area
AC-03 Old Faridabad bridge	11	Residential area
AC-04 Chandwali bridge	28	Agricultural area
AC-05 Mandkola village	41	-do-
AC-06 Janoli	46	-do-
AC-07 Ghodi	54	-do-
AC-08 Hassanpur	73	-do-
Gurgaon canal		
GC-01 Meethapur	0	Residential, waste dumping from Badarpur Industrial Area
GC-02 Mowai	5	Residential area, dumping of waste through pipeline
GC-03 Banoli	23	Residential area
GC-04 Pratapgarh	26	Residential and agricultural area, industrial waste water discharge
GC-05 Bijopur	37	Agricultural area
GC-06 Mindkola	45	-do-
GC-07 Ujjina	53	-do-

Heavy metal pollution of canals.

for washing purposes because high concentration may cause stains on the fabric. Ni concentrations exceeding 0.1 ppm can lead to several health problems like lung cancer, laryngeal cancer, dermatitis etc. (Pederson *et al.*, 1978). Lead in water, while crossing a concentration of 0.1 mg/l is harmful for fishes and human beings. Lead poisoning can cause serious nervous disorders and mental retardation in human beings. Cd concentrations throughout the canal were 0.03 ppm and such high concentrations are known to cause toxicity to fishes and to human beings as well (EPA, 1980). The concentration of Co also was higher at Tajewala and Yamuna nagar d/s. Co is known to cause neurologic abnormalities beyond 0.01 ppm (Gillies, 1978).

Agra canal : Right from the point of origin of Agra canal at Okhla barrage, the canal water is dark brown/black in colour and the upper stretch has a lot of froth and foam in it, indicating high level of pollution. The effluents of Faridabad-Ballabhgarh industrial belt (Iron works, automobiles, ceramics, pottery industries) find their way into the canal as it passes through Faridabad District. However, concentrations of the heavy metals except Fe, in Agra canal were found to be lower than that in WYC and its branch.

The iron concentration increased to 3.85 mg/l at Faridabad, whereas the permissible limits are only 0.3 mg/l. The concentration of other metals were relatively lower than that in WYC, but were still higher than the standard limits, barring Zn.

Gurgaon canal : The canal water is black in colour and is full of filth and wastes from domestic, industrial as well as agricultural sources.

While all other metal concentrations in this canal were less than that in WYC and SB, Fe concentration was the highest in this canal showing concentration of 0.76 to 5.32 mg/l. Several industries in the Faridabad district deal with iron and their effluents seem to contribute to an increase in the Fe concentration in the canal waters. The other metals except Ni and Zn were

also present in concentrations exceeding the maximum permissible limits.

Presence of abundant organic matter and biota in water bodies are reported to accumulate many of the metals, thus lowering down their free availability in water (Shafer *et al.*, 1998). Adsorption of metals even by suspended solids in water in the form of clay in water bodies reportedly, reduces the concentrations of metals in the water (Baluja *et al.*, 1983 Zhang and Huang, 1993). Bioaccumulation of metals in the eutrophicated sections of Yamuna by the biota has also been reported (Sharma *et al.*, 2000). Thus, relatively lower concentration of most of the heavy metals in the eutrophicated waters of Agra Canal and Gurgaon Canal could be because of bioaccumulation of these metals in the biota.

Interrelationships and variations in heavy metals

The inter-correlations between different heavy metals tended to differ in different canal systems. In WYC, the following metal pairs showed a significant positive correlation between each other;

Fe-Ni, $r=0.7470^*$; Fe-Pb, $r=0.6838^*$; Co-Zn, $r=0.6354^*$ ($df=3$, $* < 0.05$) in Agra canal, significant positive correlations were observed for Zn with Fe, Ni and Co and also between Fe and Co, as follows,

Fe-Zn, $r=0.7784^{**}$; Ni-Zn, $r=0.8185^{**}$; Co-Zn, $r=0.8383^{**}$; Co-Fe, $r=0.7539^{**}$ ($df=6$, $** P < 0.05$)

In Sunder branch and Gurgaon canal no two metals showed any statistically significant correlation with each other.

Variations in the heavy metal concentrations between different canals all of which originated from river Yamuna were tested for significance of difference using t-test.

Although along the route of flow of Sunder branch there are no industrial or urban

activities still the heavy metal concentrations were found to be significantly higher ($P < 0.001$) for Fe,

Ni, Pb, Co and Zn when compared with WYC, Agra canal and Gurgaon canal.

Table - 3 : Heavy metal concentrations in various canals in Haryana. (The values are mean of 3 samples).

Sample No.	Sampling station	Concentration (mg/l)					
		Fe	Ni	Pb	Cd	Co	Zn
(I) Western Yamuna Canal							
WYC-01	Tajewala head	0.14	0.13	1.39	0.03	0.25	0.30
WYC-02	Yamuna Nagar D/S	0.58	0.26	1.21	0.03	0.34	0.30
WYC-03	Karnal D/S	0.25	0.02	0.40	0.03	0.06	0.13
WYC-04	Munak	0.57	0.02	0.31	0.03	0.06	0.15
WYC-05	Haiderpur TP	2.04	0.05	0.37	0.03	0.06	0.10
Mean \pm S.E.		0.72 \pm 0.34	0.1 \pm 0.05	0.74 \pm 0.23	0.03 \pm 0.04	0.15 \pm 0.06	0.19 \pm 0.04
(II) Sunder branch							
SB-01	Safido head	0.29	0.13	1.34	0.03	0.24	0.50
SB-02	Bada Urlana	0.42	0.27	1.56	0.03	0.28	0.60
SB-03	Saifabad	0.74	0.25	1.47	0.03	0.26	0.40
SB-04	Mall Savana head	0.71	0.20	1.29	0.02	0.28	0.70
SB-05	Bodipur (Nandgarh)	0.43	0.25	1.57	0.03	0.30	1.10
SB-06	Gatauli	0.42	0.27	1.65	0.02	0.02	0.70
SB-07	100 m away from Gatauli	0.60	0.23	1.40	0.03	0.38	0.50
SB-08	Karela	0.20	0.13	1.44	0.03	0.28	0.40
SB-09	Bass	0.46	0.11	1.68	0.03	0.33	0.30
SB-10	NH-10 bridge (Mundhal)	0.06	0.20	1.52	0.03	0.27	0.20
Mean \pm S.E.		0.43 \pm 0.7	0.20 \pm 0.02	1.49 \pm 0.04	0.03 \pm 0.001	0.26 \pm 0.01	0.45 \pm 0.08
(III) Agra canal							
AC-01	Okhla head	3.33	0.02	0.37	0.01	0.01	0.05
AC-02	Faridabad 37 bridge	3.85	0.02	0.42	0.02	0.08	0.45
AC-03	Old Faridabad bridge	0.08	0.01	0.40	0.01	0.03	0.08
AC-04	Chandwali	3.96	0.09	0.34	0.02	0.06	0.28
AC-05	Mandkola	0.98	0.04	0.29	0.02	0.04	0.10
AC-06	Janoli	0.29	0.04	0.22	0.02	0.07	0.08
AC-07	Ghodi	2.11	0.05	0.38	0.02	0.04	0.51
AC-08	Hassanpur	3.60	0.11	0.40	0.02	0.08	0.74
Mean \pm S.E.		2.27 \pm 0.57	0.05 \pm 0.01	0.35 \pm 0.02	0.012 \pm 0.009	0.05 \pm 0.01	0.29 \pm 0.09
(IV) Gurgaon Canal							
GC-01	Meethapur	0.76	0.06	0.37	0.02	0.07	0.13
GC-02	Mowai	1.21	0.03	0.38	0.02	0.09	0.18
GC-03	Banoli	2.20	0.03	0.34	0.02	0.07	0.13
GC-04	Pratapgarh	5.00	0.03	0.36	0.02	0.08	0.75
GC-05	Bijopur	2.18	0.002	0.37	0.02	0.08	0.13
GC-06	Mindkola	5.32	0.06	0.43	0.02	0.07	0.15
GC-07	Ujjina	4.15	0.03	0.38	0.03	0.06	0.10
Mean \pm S.E.		2.97 \pm 0.69	0.03 \pm 0.01	0.37 \pm 0.01	0.02 \pm 0.001	0.07 \pm 0.004	0.20 \pm 0.09
Maximum Permissible Limits (mg/l) ISI		0.3	0.1*	0.1	0.01	0.01**	5.0

*WHO Standards for Ni; **USSR standards for Co.

ISI standards for these metals are not specified.

The heavy metal concentrations in Agra canal and Gurgaon canal which run parallel to each other and have similar inputs did not show any statistically significant variations ($P > 0.05$) between each other except that for Fe. In general,

Pb and Fe were the metals that differed significantly ($P < 0.01$) between different canals.

Although Agra canal and Gurgaon canal have similar pollutant inputs, yet they do not show any strong intercorrelation of the metals. This shows that even though the heavy metal inputs into

Heavy metal pollution of canals.

different canals could come from common point sources, but other factors may play a more important role in determining the solubility of metal ions and thus the inter-relationships of different metal ion species in the water. The type of micro-flora and geochemical nature of the sediments seem to play a very important role in determining the adsorption, absorption, desorption, transport, release or immobilization of heavy metal ions in the water. A direct impact of organic matter and particulate matter on the metal adsorption has been emphasized by Lo and Fung (1991). Thus, there is a need to study the heavy metal concentrations in biota and sediments for a holistic understanding of the metal contamination of the canal system.

While the presence of two or more metal species in the same effluent could result in a strong positive correlation coefficient between the two, yet there could be other factors affecting solubilization or precipitation of these metal ions, which could alter their inter-relationships. The present study indicated maximum variations in the Pb and Fe concentrations of the four canals originating from Yamuna, which suggests that these two metals are influenced more by the anthropogenic factors.

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the first of these is the fact that the human mind is not a passive recipient of impressions, but an active agent in the process of knowledge. It is not merely a mirror reflecting the world as it is, but a power which selects, interprets, and organizes the material it receives. This active role of the mind is evident in the very nature of the senses themselves. The eye, for example, does not merely receive light rays, but it interprets them as colors and forms. The ear does not merely receive sound waves, but it interprets them as tones and words. In this way, the mind is constantly at work, shaping the raw material of the senses into the coherent world we experience.

With the progress of our civilization, the human mind has become more and more active. In the past, the mind was often content to accept the traditions and authorities of its time. But now, it is more inclined to question and to seek for the truth on its own. This is a great advantage, for it allows us to discover things that our ancestors could not. But it is also a great responsibility, for it requires us to be honest and to follow the truth wherever it leads. We must not be afraid to question even the most sacred of traditions, for only in this way can we hope to reach the truth.

It is this active role of the mind that makes human knowledge so valuable. It is not merely a collection of facts and figures, but a living, growing thing that is constantly being added to and reshaped. This is why we can never stop learning. There is always more to know, and we must be ready to accept it when it comes. We must be open to new ideas and to new ways of thinking. Only in this way can we hope to keep pace with the progress of our civilization.

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Hydrobiological study of lake Mirik in Darjeeling Himalayas.

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Abstract : Some hydrobiological features of lake Mirik, situated in the Darjeeling Himalayas were studied during May to October, 2000. Water temperature showed abrupt fluctuations. The pH was generally acidic. Dissolved oxygen increased steadily with increasing rainfall and recorded highest in late August (12.6 mg l^{-1}). The gross primary productivity had a highest value of $87.50 \text{ mg C m}^{-3} \text{ hr}^{-1}$. Chlorophyceae and Cyanophyceae were identified among phytoplanktons. Zooplanktons were represented by Cladocerans and Copepods. The study revealed higher concentration of nutrients at certain pockets of the lake, which points to increasing human influences in the system, and, the water cannot serve as a scarcity alternative for drinking purpose.

Key words : Mirik lake, Limno-chemistry, Productivity, Planktons.

Introduction

A large number of natural freshwater lakes exist in the Himalayan regions, which are of great scientific and socio-economic value (Zutshi, 1985). For facilitation of commercial tourism, some reservoirs have even been artificially constructed. The Mirik Lake is an artificially constructed reservoir in the town of Mirik ($26^{\circ}54'N$ latitude and $88^{\circ}13'E$ longitude) in Darjeeling district of West Bengal.

A considerable amount of work has been carried out on different aspects of hydrobiology of the Himalayan lakes (Das *et al.*, 1969; Zutshi *et al.*, 1972; Pant *et al.*, 1985; Zutshi, 1989; Khulbe, 1992; Rawat *et al.*, 1993; Jana, 1998; Jain *et al.*, 1999). Some reports are available on the limnology of artificially constructed reservoirs in the Himalayas (Sehgal, 1989; Sugunan, 1995; Raina and Petr, 1999). Considerable hydrobiological investigations have been carried out on man-made water bodies in other parts of India (Abbasi *et al.*, 1996; Shastri and Pendse, 2001). However, waterbodies in the Darjeeling Himalayas have not been well documented. The present study was designed to obtain the present hydrobiological profile of the Mirik Lake. The Mirik town suffers from an acute shortage of drinking water during the summer months of May and June. This study

also aimed to assess the suitability of the lake water as a substitute for drinking water in time of scarcity.

Materials and Methods

The Mirik Lake is spread over 110 ha near the Krishna nagar locality in the town of Mirik at an altitude of 1767 meters above mean sea level. The inauguration of this artificial lake in 1979 led to the growth of tourism industry surrounding the lake and the subsequent rapid urbanization of Mirik has had visible impacts of disturbance on the lake and its watershed. The lake has been receiving urban effluents through a wide network of drains during the past few years.

In the present investigation, three sampling stations (S-1, S-2 and S-3) were selected for the collection of fortnightly water samples during the period of May to October, 2000. Sampling station-1 (S-1), located at the eastern part of the lake receives the effluents through drains. Sampling station-2 (S-2) located at the northern region and sampling station-3 (S-3), located at the southwestern region receives the natural runoff through the hills.

The water samples were collected from surface water at all the sites. Water temperature

was recorded by centigrade thermometer. The pH of the water samples was estimated on spot. All the other physico-chemical analysis of the water was determined following Standard Methods (APHA, 1998). The primary productivity was measured following the conventional light and dark bottle method (Gaarder and Gran, 1927). Only qualitative analysis of the phytoplanktons and zooplanktons were carried out. Correlation between the different physico-chemical parameters and plankton productivity were calculated following Karl Pearson's method (Palanichamy and Manoharan, 1990) and the corresponding significant test were performed to find out the level of significance.

Results and Discussion

The results of the water quality and hydrobiological analysis are presented in Table-1. The water temperature ranged between 20°C and 31°C during the period of study. The variation in temperature was significant between the summer and rainy seasons. Similar fluctuation of temperature between seasons was also reported by Jain *et al.* (1999). Specific conductivity values were usually higher in S-1, which had a mean of 0.42 mmhos cm⁻¹, than in other sites, which could be attributed to the greater ionic concentration of the inlet flow. The mean values of specific conductivity in S-2 and S-3 stood at 0.35 and 0.26

Table – 1 : Summary of the hydrobiological features of the Mirik Lake.

Parameters	S-1			S-2			S-3		
	Range	Mean	±S.D.	Range	Mean	±S.D.	Range	Mean	±S.D.
Water temperature (°C)	20.0-31.0	24.1	±2.70	20.0-31.0	24.1	±2.70	20.0-31.0	24.1	±2.70
Hydrogen ion concentration (pH)	5.8-6.8	6.22	±0.46	6.1-6.8	6.34	±0.31	6.3-6.9	6.46	±0.42
Specific conductivity (mmhos cm ⁻¹)	0.20-0.84	0.42	±0.16	0.10-0.70	0.35	±0.19	0.10-0.72	0.26	±0.18
Dissolved oxygen (mg l ⁻¹)	5.2-12.4	7.24	±2.68	5.4-11.8	7.40	±1.92	5.6-12.6	7.64	±2.08
Free carbon dioxide (mg l ⁻¹)	5.2-6.4	5.62	±0.82	5.6-6.2	5.90	±0.30	5.0-5.8	5.38	±0.41
Total alkalinity (mg l ⁻¹)	28.0-46.0	38.08	±4.26	28.0-42.0	36.12	±3.27	26.0-36.0	31.49	±2.84
Total hardness (mg l ⁻¹)	10.4-26.4	16.24	±4.25	10.2-24.0	15.42	±3.82	8.8-20.2	13.11	±2.92
Chloride ion (mg l ⁻¹)	15.8-26.8	22.02	±2.95	16.2-24.8	19.60	±2.51	12.2-20.4	16.60	±2.97
Phosphate-P (mg l ⁻¹)	0.03-0.14	0.07	±0.04	0.02-0.10	0.04	±0.03	0.03-0.08	0.04	±0.02
Ammonium-N (mg l ⁻¹)	0.012-0.072	0.046	±0.024	0.010-0.052	0.032	±0.018	0.006-0.036	0.022	±0.013
Nitrite-N (mg l ⁻¹)	0.008-0.032	0.017	±0.010	0.006-0.024	0.013	±0.006	0.002-0.016	0.008	±0.005
Nitrate-N (mg l ⁻¹)	0.06-0.34	0.18	±0.08	0.04-0.28	0.15	±0.09	0.04-0.18	0.09	±0.06
Primary productivity									
Gross Primary Productivity (GPP) (mg C m ⁻³ hr ⁻¹)	25.5-67.5	46.25	±14.28	37.5-87.5	55.25	±16.05	41.25-87.5	62.50	±16.64
Net Primary Productivity (NPP) (mg C m ⁻³ hr ⁻¹)	20.25-58.25	32.24	±12.06	30.75-62.75	42.25	±10.38	30.75-60.0	44.50	±12.68

S.D. = Standard Deviation; (n = 11 for GPP and NPP, and for all other parameters, n = 13).

mmhos cm⁻¹, respectively. The water of the lake was generally acidic as can be observed from the pH values (Table-1). The rainwater however, played its part in neutralizing the acidic level and

the highest pH value during our study was recorded in August (6.9). The acidic nature of Himalayan lakes has also been reported by Zutshi *et al.* (1972) and Rawat *et al.* (1993).

A positive correlation was observed between water temperature and dissolved oxygen (DO) (Table-2). The DO varied between 5.2-12.6 mg l^{-1} in the lake water. The values showed an increasing tendency from early May onwards, reached the peak at the end of August, and decreased thereafter. Higher DO values during the monsoon may be due to turbulence and oxygenation resulting from high rainfall and

mixing up of the well aerated run of stream coming from the surrounding hills. The free CO_2 content showed no remarkable fluctuation (Table-1). Correlation studies showed highly negative relation between pH and free carbon dioxide [$r = (-) 0.684$]. Again, the free carbon dioxide recorded a highly significant negative relation with dissolved oxygen [$r = (-) 0.620$]. Therefore, it may be possible that high dissolved oxygen concentration

Table - 2 : Pearson's correlation coefficients for physico-chemical characteristics and plankton productivity of the Mirik lake ($n = 11$, d.f. = 9 for gross primary productivity, and for all other parameters, $n = 13$, d.f. = 11).

Parameters	Temperature	pH	Conductivity	DO	Free CO_2	Alkalinity	Hardness	Chloride	$\text{NH}_4\text{-N}$	$\text{NO}_2\text{-N}$	$\text{NO}_3\text{-N}$	$\text{PO}_4\text{-P}$	GPP
Temperature	-	0.206	0.142	0.218	-0.331	-0.618	-0.314	-0.229	-0.162	-0.287	0.371	0.102	0.214
pH	NS	-	-0.270	0.610	-0.684	-0.756	-0.226	-0.686	-0.372	-0.336	0.226	0.284	0.216
Conductivity	NS	NS	-	-0.194	0.306	0.225	0.160	0.462	0.247	0.119	-0.215	0.068	0.263
DO	NS	0.05	NS	-	-0.620	-0.643	-0.414	-0.422	-0.461	-0.421	0.309	0.396	0.439
Free CO_2	NS	0.05	NS	0.05	-	0.692	0.273	0.428	0.372	0.255	-0.241	-0.313	-0.283
Alkalinity	0.05	0.01	NS	0.05	0.05	-	0.614	0.627	0.323	0.314	-0.210	-0.281	-0.473
Hardness	NS	NS	NS	NS	NS	0.05	-	0.380	0.257	0.301	-0.238	-0.260	-0.262
Chloride	NS	0.05	NS	NS	NS	0.05	NS	-	0.312	0.399	-0.407	-0.056	-0.372
$\text{NH}_4\text{-N}$	NS	NS	NS	NS	NS	NS	NS	NS	-	0.327	-0.232	-0.377	-0.334
$\text{NO}_2\text{-N}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	-0.218	-0.231	-0.290
$\text{NO}_3\text{-N}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	0.152	0.406
$\text{PO}_4\text{-P}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	0.208
GPP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-

NS = Not Significant; Lower matrix = Probability values; Upper matrix = Correlation coefficients.

results in an increased pH value and vice-versa, possibly due to comparatively high photosynthetic activity during this period. The free CO_2 also recorded a significantly positive correlation with total alkalinity (Table-2).

The total alkalinity here refers to bicarbonate alkalinity, as carbonate was absent in the lake water. Low alkalinity values were observed during the rainy season and relatively more during the pre and post-monsoon period. At S-1, the total alkalinity recorded 28mg l^{-1} at the end of August against a total average of 38.08mg l^{-1} . Adebisi (1980) showed alkalinity to be inversely related to water level. Lower alkalinity in the rainy seasons was also reported by Dhanapakiam *et al.* (1999), Shastri and Pendse (2001). The total alkalinity showed a strong negative correlation with pH and DO but a strong positive correlation with chloride (Table- 2). The total hardness of the

surface water varied from $8.8\text{-}26.4\text{mg l}^{-1}$, the value fluctuating positively in accordance to the total alkalinity ($r = 0.614$). According to Kannan (1991), water with a hardness value less than 60mg l^{-1} is soft. Hence, the lake water can be regarded as soft. Sehgal (1989) and Sugunan (1995) reported low level of total hardness in the Pong reservoir.

Chloride concentration varied from $12.2\text{-}26.8\text{mg l}^{-1}$ in the lake water. Chloride concentration indicates the presence of organic waste, primarily of animal origin (Thresh *et al.*, 1949). Munawar (1970) further suggested that higher concentration of chloride in the water is an index of pollution of animal origin and there is a direct relation between chloride concentration and pollution level. Low concentration of chloride ion in the lake water, particularly in the monsoon indicates, there is low amount of organic waste of

animal origin during the rainy season. Ground condition in Mirik revealed that the municipality drains carried more of fresh rainwater than sewage effluents into the lake during the monsoon. Sehgal (1989) recorded very low level of chloride in the Pong reservoir. Shastri and Pendse (2001) also reported lower values of chloride during rainy season.

The Ammonium-N values ranged from 0.006-0.072 mg l^{-1} in the lake water. The low $\text{NH}_4\text{-N}$ concentrations may be due to the fact, that aquatic autotrophs rapidly utilize ammonium ions preferring these to nitrates; accordingly, $\text{NH}_4\text{-N}$ does not reach a harmful concentration. The $\text{NH}_4\text{-N}$ is negatively correlated to dissolved oxygen (Table-2). Jana and Barat (1984) observed similar relation between DO and $\text{NH}_4\text{-N}$. At S-1, the $\text{NH}_4\text{-N}$ values recorded higher throughout the period of study, indicating sewage contamination as, ammoniacal nitrogen owes itself mostly to animal excreta. The Nitrite-N concentrations were found out to be quite low. The maximum concentration of $\text{NO}_2\text{-N}$ (0.032 mg l^{-1}) was recorded in early May at S-1. The minimum value was recorded at S-3 (0.002 mg l^{-1}) in September. The much higher mean value of $\text{NO}_2\text{-N}$ in S-1 (0.017 mg l^{-1}) in comparison to that of S-3 (0.008 mg l^{-1}) points to the higher concentration of effluents in S-1. Higher values of $\text{NO}_2\text{-N}$ at S-1 may also be due to oxidation of ammonia. Similar observations have been reported by Kapila and Patel (1999). Lower values of the nutrient during the rainy season could be attributed to a dilution effect.

The Nitrate-N is one of the most oxidisable forms of nitrogen and is an essential plant nutrient. Due to its higher mobility, its concentration in fresh water, apart from autochthonous production and utilization by plants, is also regulated by waste loading, agricultural runoff and ground water inputs. Thus, $\text{NO}_3\text{-N}$ concentration is associated with rainwater runoff, sewage and sullage discharge. The $\text{NO}_3\text{-N}$ ranged from 0.04-0.34 mg l^{-1} in the lake water. Sugunan (1995) reported much lower level of $\text{NO}_3\text{-N}$ in the Govind Sagar reservoir. The highest

concentrations of the nutrient were recorded after the onset of rains, probably by the transport of nutrients from the watershed areas with the runoff water. In S-1, the $\text{NO}_3\text{-N}$ recorded relatively higher throughout the period of study. These points to the sewage contamination via the drains. Alderfer and Lovelace (1977) remarked that inorganic nitrogen above 0.03 mg l^{-1} stimulates algal growth to such an extent that the water may not be suitable for human consumption. From this aspect, the lake water is not healthy for drinking purpose. Pant *et al.* (1985) reported on the rising level of nitrogen in lake Nainital due to increasing human influences.

Throughout the investigation period, the Phosphate-P concentration was found out to be low. The maximum value of 0.14 mg l^{-1} was obtained from S-1 in July. The minimum value (0.02 mg l^{-1}) was recorded at S-2 in October. Phosphate is considered amongst the primary limiting nutrients in ponds and lakes (Schindler, 1971). Low values of phosphate have been reported from various Himalayan lakes and reservoirs (Raina and Petr, 1999). Higher values of this nutrient at S-1 compared to the other sites may be due to discharge of domestic sewage into the water. Similar increase in $\text{PO}_4\text{-P}$ at the point of sewage discharge was reported by Kapila and Patel (1999).

The overall mean of gross primary productivity in S-3 (62.50 $\text{mg cm}^{-3} \text{ hr}^{-1}$) was higher than S-1 (46.25 $\text{mg cm}^{-3} \text{ hr}^{-1}$). According to a report, the productivity of the Dal Lake in Kashmir was found out to be in the range of 20.99-31.23 $\text{mg cm}^{-3} \text{ hr}^{-1}$ (Anon, 1977). The productivity of the Mirik Lake seems to be higher. Both gross primary productivity and net primary productivity were found out to be low on cloudy days. Romaine and Boyd (1979) also showed that cloudy days cause a decrease in photosynthetic rates. The net primary productivity was found out to be 69.70%, 76.47% and 71.20% of the gross primary productivity in the S-1, S-2 and S-3, respectively. The gross primary productivity showed a positive correlation with dissolved oxygen ($r = 0.439$) and Nitrate

Hydrobiology of Lake Mirik.

nitrogen ($r = 0.406$). Datta *et al.* (1984) also established a positive correlation between Nitrate nitrogen and gross production.

An attempt was made to study the prominent groups of phytoplanktons and zooplanktons present in the lake water. Among the phytoplanktons, Chlorophyceae and Cyanophyceae were the prominent groups as *Spirogyra*, *Closterium*, *Phormidium*, *Scenedesmus*, *Stigeoclonium*, *Ulothrix* and *Oscillatoria* could be identified, which clearly indicate that the lake water is polluted. Cladocerans and Copepods mostly represented the zooplankton community. *Cyclops* was the most abundant zooplankton. Others among copepods were *Phyllodiaptomus*. Among the cladocerans, *Moina*, *Daphnia* and *Bosmina* were recorded.

In the present investigation, certain pockets of the lake (S-1) appeared to be more disturbed by external influences compared to the other zones. S-3 seemed to be least polluted. The contaminants that the lake received were clearly manifested in the results, although the values were diluted due to heavy rainfall in the monsoons. Based on the limnological investigations, and planktons identified, the water does not seem fit for domestic use and cannot serve as a scarcity alternative for drinking water. The results obtained in the present study shall be helpful in the future management of the Mirik Lake and proper development of Mirik town as a whole.

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Effect of cybil on reproductive success of wild *Drosophila melanogaster*.

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Abstract : Cybil (a synthetic pyrethroid) was used to assess its impact on the reproductive success in F_1 and F_2 generations of wild *Drosophila melanogaster*. The LC_{50} has been estimated as $0.0267 \mu\text{l}/100 \text{ ml}$ food. Reproductive success has been found to be affected in addition to fecundity and pupation following toxicity of cybil.

Key words : Cybil, Reproductive success, *Drosophila melanogaster*.

Introduction

The pyrethroids have broad spectrum activity and low mammalian toxicity. These do not affect the non-target species. They are rapidly metabolized and practically no residues are left behind.

Cybil (25% active ingredient of cypermethrin) has been used on a variety of crop plants because of its high insecticidal activity coupled with its relative safety to mammals. Toxicity studies have been made on *Drosophila melanogaster* because of its ease in rearing and short life span.

Earlier workers reveal that many pesticides have been tested on *Drosophila melanogaster* (Srivastava and Chatteraj, 1979; Vasudev and Krishnamurthy, 1982-83; Riaz Mahmood *et al.*, 1990; Basheer *et al.*, 1999). However, the effect of synthetic pyrethroids has not been found on *Drosophila melanogaster*. Hence, toxicity of cybil has been evaluated in the present study.

Materials and Methods

A pure strain of *Drosophila melanogaster* (Meigen, wild type) was obtained from *Drosophila* stock center (School of Life Sciences, Devi Ahilya Vishwavidyalaya, Khandwa Road Campus, Indore). The pure culture was maintained under the laboratory conditions at a temperature $25 \pm 5^\circ\text{C}$

and on a standard food medium consisting of distilled water 360 ml, agar 2g, corn flour 17g, sugar 12g, yeast 3 g, nepagin 1 g, propionic acid 1 ml and 70% alcohol 1 ml.

Toxicity analysis : Flies used for experimentation were used after 5-6 generations. LC_{50} was first calculated by testing different quantities of cybil, subsequently sub-lethal dose of $0.015 \mu\text{l}/100 \text{ ml}$ food was selected. In order to determine the effectiveness of the test compound different cross-combinations ($T \text{ } \text{f} \times UT \text{ } \text{m}$, $T \text{ } \text{f} \times T \text{ } \text{m}$, $UT \text{ } \text{f} \times T \text{ } \text{m}$) were examined against the control set ($UT \text{ } \text{f} \times UT \text{ } \text{m}$) where T = treated and UT = untreated. The male and female flies were in the ratio of 1 : 1 and were separated under ether anesthesia previously. Eggs were collected by Delcour procedure (Delcour, 1969) to determine fecundity. Eggs of each set were allowed to undergo development in the culture bottles at $25 \pm 5^\circ\text{C}$. Observations were made on pupation and adult emergence in both F_1 and F_2 generations. The flies emerged were counted and sexed every day from the first to the last day of eclosion.

Statistical analysis : The collected data were analysed statistically by log-dose/probit regression line method. (Finney, 1971). The statistical calculations of mean, standard deviation and standard error were based on the biological statistics by Fisher and Yates (1948). The test of significance was made using simple t-test. Significance by Fisher's formula was used to

calculate the statistical significance between the control and the treated sets.

Results and Discussion

Observations reveal that the survival rate decreases with the increasing dose of the test chemical which is in accordance to Laamanen *et al.* (1976), Vasudev and Krishnamurthy (1978) using amitrole and dithane M-45 on *Drosophila*

melanogaster and also gains support of Bhagat *et al.* (1997) and Basheer *et al.* (1999).

The fecundity (Table 1 and 2) of the treated sets in both F₁ and F₂ generations of the form has been observed to show reduction, which is in conformity with Thakur and Kumar (1984). This reduction may be due to the inhibitory effects of the compound on the gonadal development. The present finding is in accordance to Santhi *et al.* (1993), Omer and Leigh (1995).

Table – 1 : Percent reproductive success of experimental wild *Drosophila melanogaster* in F₁ generation.

S. No.	Sets	Fecundity (Mean \pm SE)	Percent reproductive success (Mean \pm SE)
1	T ♀ \times UT ♂	241.66 \pm 8.89	61.88 \pm 0.96
2	T ♀ \times T ♂	227.33 \pm 5.30	43.23 \pm 0.70
3	UT ♀ \times T ♂	252.66 \pm 9.20	70.26 \pm 1.07
4	UT ♀ \times UT ♂	267.33 \pm 12.45	92.72 \pm 0.93

Table – 2 : Percent reproductive success of experimental wild *Drosophila melanogaster* in F₂ generation.

S. No.	Sets	Fecundity (Mean \pm SE)	Percent reproductive success (Mean \pm SE)
1	T ♀ \times UT ♂	231.66 \pm 7.36	41.32 \pm 1.51
2	T ♀ \times T ♂	213.33 \pm 7.36	25.27 \pm 0.68
3	UT ♀ \times T ♂	243.33 \pm 7.36	57.18 \pm 1.95
4	UT ♀ \times UT ♂	253.33 \pm 10.80	81.52 \pm 1.04

Further, decrease in pupation is because of the fact that larval forms contain many dividing cells which are sensitive to the somatic damage caused by the chemical and thus leads to lesser pupation and gains support from finding of Laamanen *et al.* (1976) and Srivastava and Chatteraj (1979).

Tables 1 and 2 reveal that the reproductive success has been reduced in both generations as compared to the control. Assessment of reproductive success gives an indication of increase or decrease of population. The reduction in reproductive success is due to the affect of the chemical upon various stages of development and it has been found that it mainly affects the apolytic process thus keeping a check on the population. Similar reduction was observed by Banerjee *et al.*

(1993), Basheer *et al.* (1999). Effects on reproductive success in different insects have also been reported by Boetel *et al.* (1998) and Martin *et al.* (1998).

The observed effects on the reproductive success in the two generations of wild *Drosophila melanogaster* give clear indications that the test chemical affects fecundity, pupation and causes an impairment in apolytic process.

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Antimicrobial resistance among enteric bacteria, isolated from runoff of the Gangotri glacier, western Himalaya India.

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Abstract : An attempt has been made to study antimicrobial resistance among the coliforms, faecal coliforms and faecal streptococci isolated from the runoff of the Gangotri glacier. The number of multiple antibiotic resistances (MAR) coliform isolates increases from upper stretch (33.33%) to lower stretch (83.33%). All faecal coliforms (100%) and faecal streptococci (100%) of lower stretch of study area showed multiple antibiotic resistances. Among coliforms, the value of Antibiotic resistance index (ARI) was found maximum in lower stretch (0.34) followed by middle stretch (0.29) and upper stretch (0.25).

Key words : Gangotri glacier, Antibiotics, Bacterial resistance, Coliforms.

Introduction

Gangotri glacier, around 30 km in length covering an area of 143 km², flowing North West, is one of the largest valley type glacier in the Western Himalaya (Singh and Yadav, 2000). Himalayan glaciers are an important ever renewing source of fresh water for the millions, living in the plains of northern and eastern India. The incidence of antibiotic-resistant bacteria in aquatic environments has increased dramatically as a consequence of the widespread use of antibiotics by human. This increase has resulted from a variety of factors, perhaps the most important of which is the selection for resistant strains and the ability of such strains to exchange plasmids encoding resistance (Licht *et al.*, 2002). The continuous exposure of microbial population to antibiotics (De Vicente, *et al.*, 1990), heavy metals (Barkay, *et al.*, 1985) selects for resistant strains. The number of species and the strains of pathogenic and commensal bacteria resistant to antibiotics and the number of antibiotics to which they are resistant has increased virtually monotonically worldwide (Iversen, *et al.*, 2002; Borgen *et al.*, 2002). In addition, resistant bacteria are known as useful bioindicators for polluted habitats (Margesin and Schinner, 1996). Enteric

bacteria i.e. coliform, faecal coliforms and faecal streptococci are widely used indicators for bacteriological quality of water (Kistemann *et al.*, 2002; Pathak and Gopal, 2001). Recent reports indicate that antimicrobial resistance among enteric bacteria is on the rise (Galland *et al.*, 2001; Schroeder *et al.*, 2002). Thus studies are required to focus on the association of bacterial mechanisms of antibiotic resistance and data bases are needed on antibacterial resistance. Research is also needed to explore the best strategies for the optimal use of antibiotics and to estimate the effect of antibiotic exposure of the risk of emerging resistance, as well as on the dynamic of diffusion in the population (Bax *et al.*, 1998 and Levin *et al.*, 1998). An attempt has been made to study antimicrobial resistance among the coliforms faecal coliforms and faecal streptococci isolated from the runoff of the Gangotri glacier. Baseline data generated on resistance profile of some important antibiotics among enteric bacteria may serve as a standard comparison for other glaciers including lower Ganges.

Materials and Methods

Water samples from 21 different sites of runoff of the Gangotri glacier covering an stretch

of 250 km from Gangotri to Haridwar was collected in winter, summer and monsoon seasons (Fig. 1) in sterile glass bottles, transported on ice to the base laboratory and processed within 6-8 hour of collection. Study area from Gangotri to Haridwar is divided into three parts. i Upper stretch i.e. Gangotri glacier area; ii Middle stretch; and iii Lower stretch. In this upper stretch i.e.

Gangotri glacier area constitutes five sampling sites viz. G_1 , G_2 , G_3 , G_4 and G_5 , middle stretch constitutes eight sampling sites viz. Gangotri, Dharali, Gangnani, Bhatwari, Maneri, Uttarkashi, Dunda and Dharasu. Lower stretch also constitutes eight sampling sites viz. Tehri, Devprayag US, Devprayag DS, Kaudiyala, Marine drive, Shivepari, Rishikesh and Haridwar.

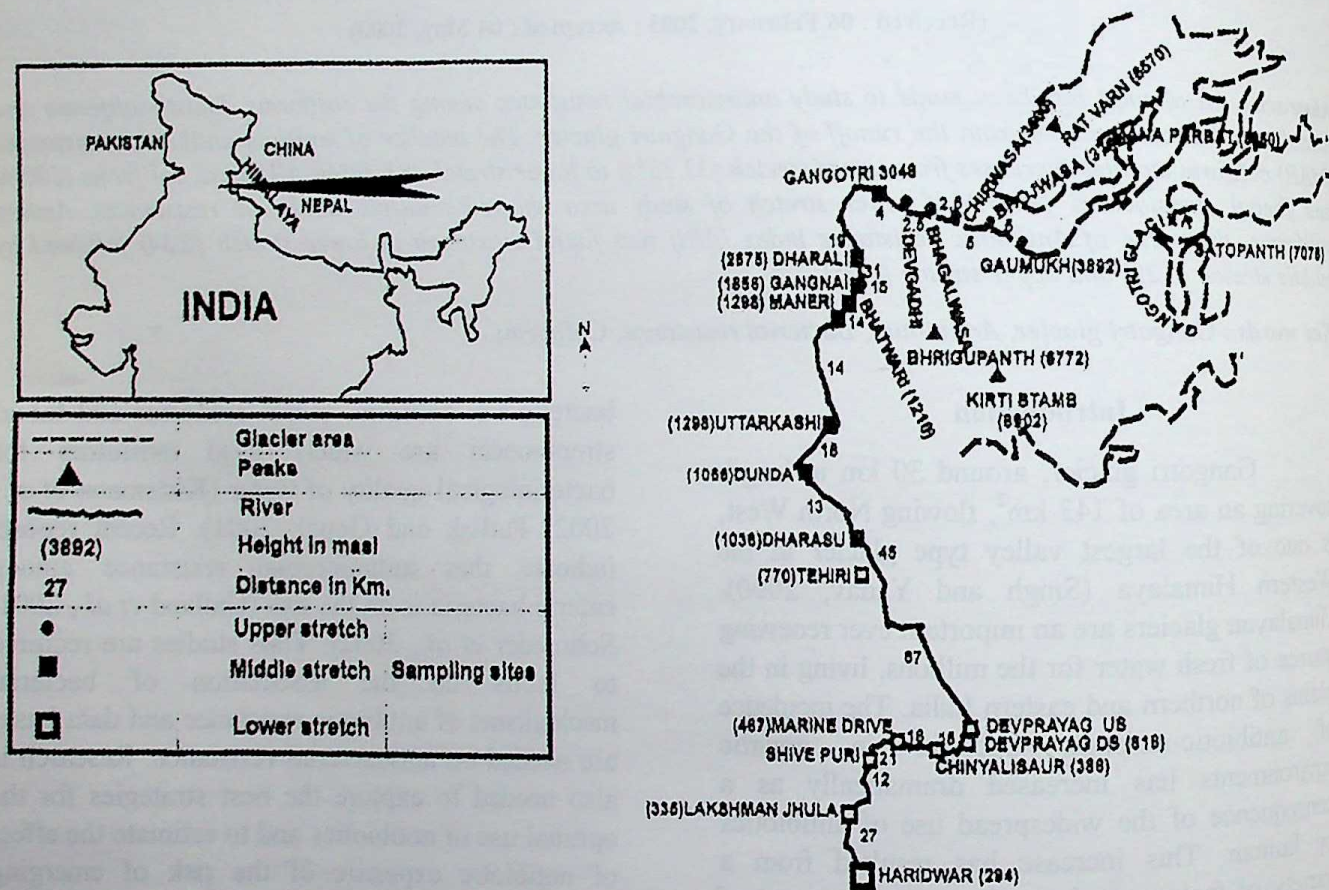


Fig. 1 : Location map of the study area.

Coliforms, faecal coliforms and faecal streptococci were isolated from different sampling sites from positive coliform, faecal coliform and faecal streptococci test-by using MPN method (APHA, 1998). Coliforms were detected by inoculation of samples in Mac-Conkey broth and incubated at $37 \pm 0.5^\circ\text{C}$ for 48 hours. The positive tubes were subcultured into brilliant green bile broth (BGBB) and incubated at $44.5 \pm 0.5^\circ\text{C}$. Gas production in BGBB at $44.5 \pm 0.5^\circ\text{C}$ were used for the detection of faecal coliform after 48 hours of incubation. Faecal streptococci were detected by inoculation of water samples into azide dextrose

broth and incubation at $37 \pm 0.5^\circ\text{C}$ for 24-48 hours. Purified colonies were obtained by repeated subculturing of the positive tubes of the total coliform, faecal coliform and faecal streptococci on nutrient agar plates. The scheme of Cowan and Steel (1998) was followed for the identification of coliforms, faecal coliforms and faecal streptococci.

The disk diffusion method was performed to screen organisms susceptible to different antibiotics (Bauer *et al.*, 1966). Isolates of coliforms, faecal coliform and faecal streptococci collected from different sampling sites were

Antimicrobial resistance among enteric bacteria.

screened for their resistance pattern for the following antibiotics ($\mu\text{g/disk}$). 1. Polymyxin-B (50), 2. Nitrofurantoin (30), 3. Ampicillin (25), 4. Colistin (25), 5. Nalidixic acid (30), 6. Streptomycin (25), 7. Tetracycline (30), 8. Chloramphenicol (30), 9. Kanamycin (30), 10. Gentamycin (10).

The antibiotic resistance index (ARI) was calculated by Hinton *et al.* (1985).

$$\text{ARI} = Y/nx,$$

where,

Y = is the total number of resistance scored.

n = is the number of isolates.

x = is the number of antibiotics tested

Results and Discussion

Sixty-three coliforms were isolated from twenty-one different sampling sites during winter, summer and monsoon season. All isolates were screened for their susceptibility to different antibiotics. Coliforms were found predominantly resistant to gentamycin (57.14%) followed by tetracycline (52.38%), colistin (46.03%), ampicillin (42.85%) and polymyxin-B (36.50%).

Moderate resistance was observed for nitrofurantoin (26.98%) and chloramphenicol (20.63%). Resistance to kanamycin was observed in very few (17.46%) isolates. All coliforms were found sensitive to nalidixic acid and streptomycin. Most of the isolates of faecal coliforms showed resistance to tetracycline (73.01%) followed by polymyxin-B (60.31%), colistin (53.96%), kanamycin (31.74%), chloramphenicol (30.15%) and gentamycin (30.15%). Moderate resistance was observed for streptomycin (22.22%) and ampicillin (14.28%). Isolates showed lower resistance to nitrofurantoin (7.93%) and nalidixic acid (4.76%). Majority of the isolates of faecal streptococci were found resistant to ampicillin (53.96%) followed by streptomycin (47.61%), polymyxin-B (46.03%), kanamycin (46.03%), tetracycline (41.61%), colistin (33.33%), nalidixic acid (25.39%), gentamycin (25.39%), nitrofurantoin (23.80%) and chloramphenicol (19.04%) (Fig. 2).

Among all the isolates of coliforms studied majority of the isolates were found resistant to different antibiotics in lower stretch with a gradual decrease in middle and upper stretch.

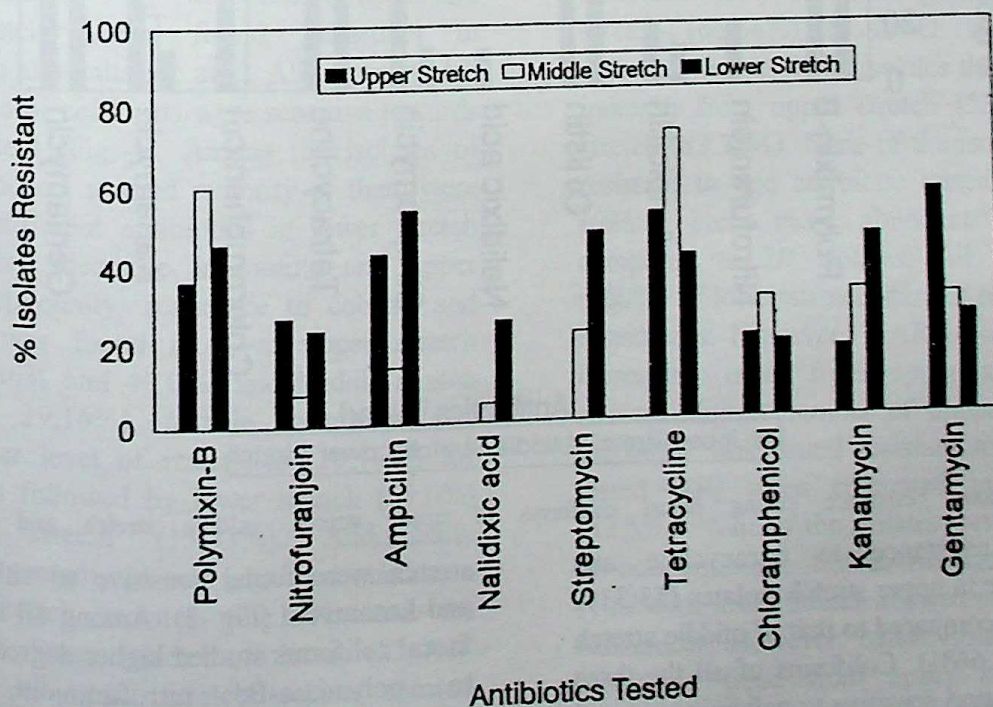


Fig. 2 : Antibiotic resistance among the isolates of coliforms, faecal coliforms and faecal streptococci.

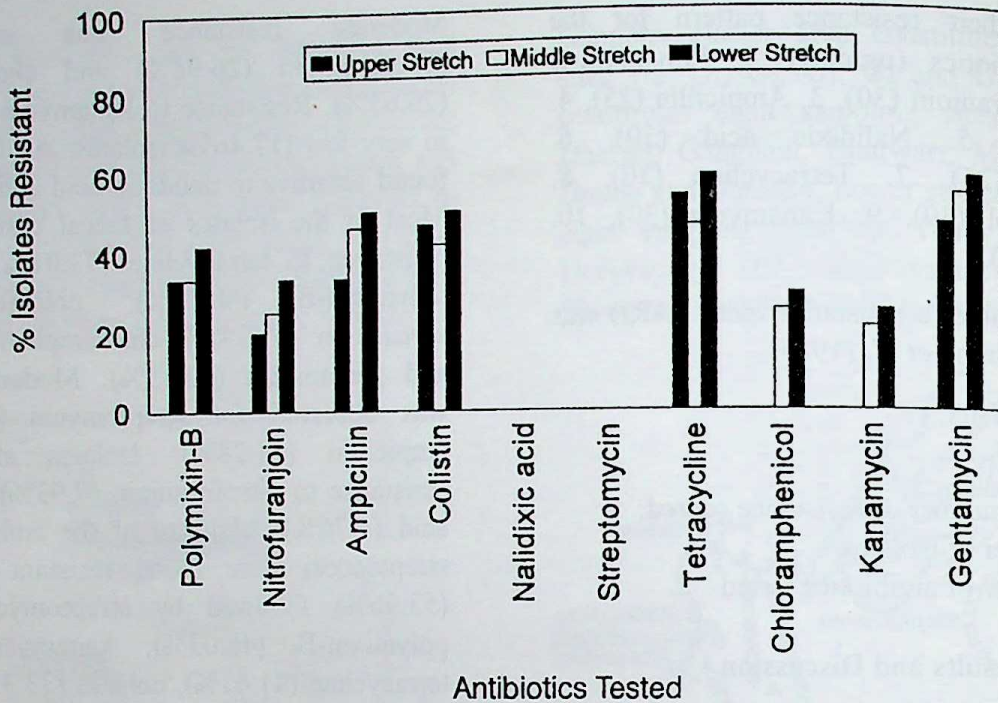


Fig. 3 : Antibiotic resistance among coliforms of upper stretch, middle stretch and lower stretch.

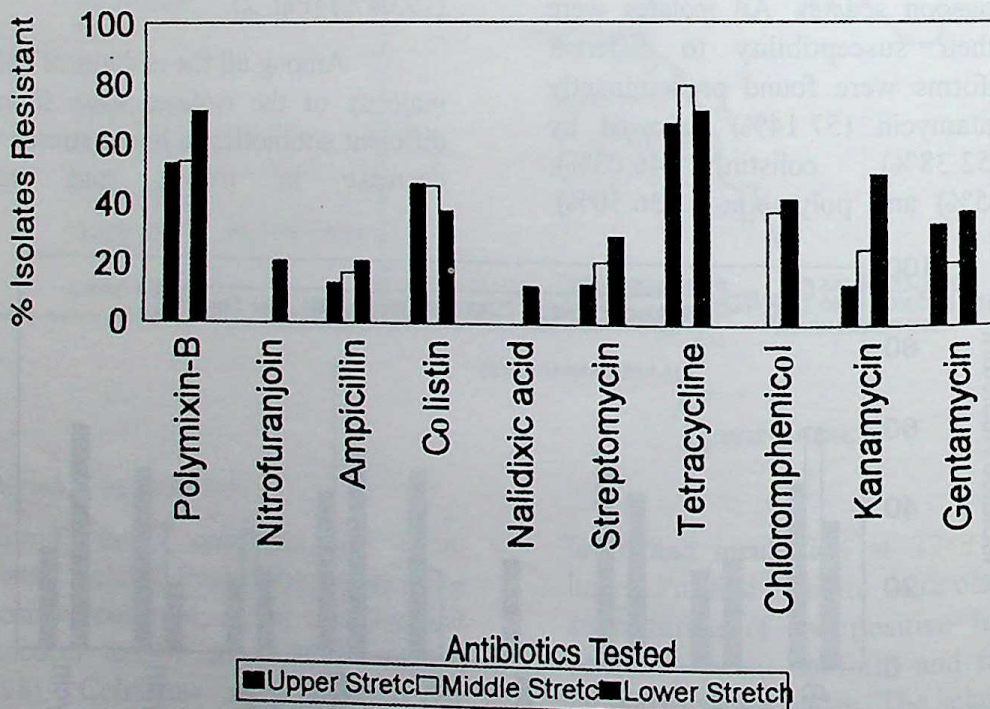


Fig. 4 : Antibiotic resistance among faecal coliforms of upper stretch, middle stretch and lower stretch. Exceptionally, resistance to tetracycline and colistin was more in upper stretch isolates (53.33% and 46.66%) as compared to that of middle stretch (45.83% and 41.66%). Coliforms of all the three stretches were found sensitive to nalidixic acid and streptomycin. Additionally, all isolates of upper stretch were found sensitive to chloramphenicol and kanamycin (Fig.-3). Among all the isolates of faecal coliforms studied higher degree of resistance to polymixin-B, nitrofurantoin, ampicillin, nalidixic acid, streptomycin, chloramphenicol and kanamycin were observed in lower stretch isolates.

with a gradual decrease in middle and upper stretch. However, resistance to gentamycin was more in upper stretch (33.33%) when compared to

middle stretch (20.83%). Further, resistance to tetracycline was more abundant in middle stretch isolates (79.16%) with a gradual decrease in lower

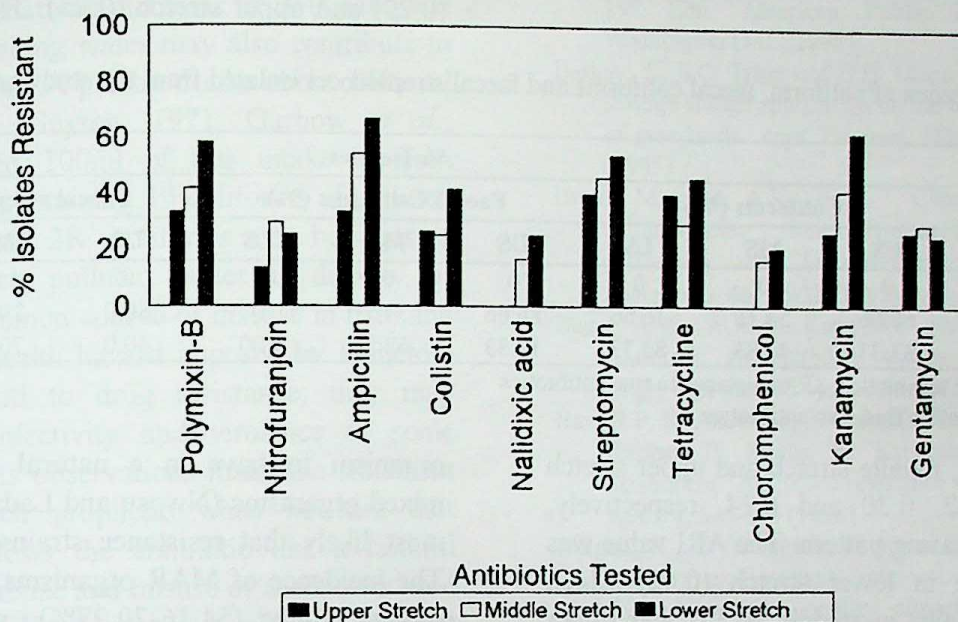


Fig. 5 : Antibiotic resistance among faecal streptococci of upper stretch, middle stretch and lower stretch.

stretch (70.83%) and upper stretch (66.66%), whereas, for colistin maximum resistance was observed among upper stretch isolates (46.66%) with a gradual decrease in middle stretch (45.83%) and lower stretch 37.5%). All isolates of upper and middle stretch were found sensitive to nitrofurantoin and nalidixic acid. All upper stretch isolates of faecal coliforms were sensitive towards chloramphenicol (Fig.-4). Among the isolates of faecal streptococci studied majority of them were resistant to different antibiotics in lower stretch with a gradual decrease in middle and upper stretch. Exceptionally, resistance to colistin and tetracycline was found more in upper stretch isolates (26.66% and 40.0%) and Middle stretch (25.0% and 29.16%). Middle stretch isolates showed higher level of resistance (29.16%) for nitrofurantoin followed by lower stretch (25.0%) and upper stretch (13.33%). Additionally resistance to gentamycin was observed maximum in middle stretch isolates (29.16%) and the minimum in lower stretch isolates (25.0%). Furthermore, all upper stretch isolates showed sensitivity towards nalidixic acid and chloramphenicol (Fig.-5).

The incidence of MAR (multiple antibiotic resistant) isolates isolated from different sampling stations of all the three stretches are given in Table-1. The number of coliforms resistant to two antibiotic (2R) decreased gradually from upper stretch (66.66%) to lower stretch (16.66%), whereas, among MAR isolates there was a gradual increase from upper stretch (33.33%) to lower stretch (83.33%). None of the isolates were found resistant to one antibiotic tested. Overall, MAR isolates were more abundant (57.14%) when compared to 2R isolates. All faecal coliforms (100%) of lower stretch showed multiple antibiotic resistances. However, MAR isolates exhibited an increasing trend from upper stretch to lower stretch. Higher number of upper stretch isolates (86.66%) was found resistant to two antibiotics tested (2R) when compared to middle stretch (37.5%). None of the isolates were found resistant to one antibiotic tested. All faecal streptococci (100%) of lower stretch showed multiple antibiotic resistances. However, MAR isolates exhibited an increasing trend from upper stretch to lower stretch. Higher number of upper stretch isolates (60.0%) were found resistant to two antibiotics

(2R) tested when compared to that of middle stretch (29.16%). None of the isolates of any stretch were found resistant to one antibiotic (1R) tested.

Among coliforms, the value of ARI (antibiotic resistance index) was found maximum in lower stretch (0.34) followed by middle stretch (0.29) and upper stretch (0.25). The value of ARI

Table - 1 : MAR index of coliform, faecal coliforms and faecal streptococci isolated from the study area.

MAR Index	% Isolates								
	Coliforms (%)			Faecal Coliforms (%)			Faecal streptococci (%)		
	US	MS	LS	US	MS	LS	US	MS	LS
1R	0	0	0	0	0	0	0	0	0
2R	66.66	54.16	16.66	86.66	37.5	0	60.0	29.16	0
MAR	33.33	45.83	83.33	13.33	62.5	100	40.0	70.83	100

1R=resistance to one antibiotics; 2R=resistance to two antibiotics.
MAR=resistance to more than two antibiotics.

for lower stretch, middle stretch and upper stretch isolates are 0.42, 0.30 and 0.24, respectively, reflecting a decreasing pattern. The ARI value was found maximum in lower stretch (0.42) which descended gradually in middle (0.35) and upper stretch (0.24).

Antibiotic response of bacteria is useful in understanding the scope and significance of antibiotic resistance not only in clinical isolates but also in environmental isolates. All the isolates of coliforms, faecal coliforms and faecal streptococci show rising trend of resistance from upper stretch to lower stretch. The differences in resistance profiles in this ecological study clearly reflect the differences in selection pressure in the investigated geographical location. The increased prevalence and dissemination of bacterial antimicrobial resistance is a natural expression of evolution and bacterial genetics (Levy, 2000). The more a particular antimicrobial agent is used, the greater the chance of microorganisms developing resistance. This is generally attributed to the indiscriminate use of antibiotics which encourage the spread of transmissible plasmids carrying antibiotic resistance among susceptible populations of bacteria. Basu and Paul (1999) also reported that the multiple antibiotic resistances of some of the isolates indicate the possible acquisition of a plasmid-conferred antibiotic resistance factor by the specific isolates. Moreover, resistance to antibiotics may be a desirable characteristic for an

organism to have in a natural environment of mixed organisms (Nwosu and Ladapo, 1999). It is most likely that resistance strains had R-factors. The incidence of MAR organisms is higher in the present study (54.16-70.27%), when compared with earlier investigations (6.1-31.4%) (Ramteke, 1997; Gaur *et al.*, 1992) clearly indicating the rising trend in the spread of drug resistant bacteria. The enhancement of MAR bacteria observed in Bhagirathi water system may be of health significance. Human infections caused by such organisms could be difficult to treat with drugs. In our study, the level of resistance to antibiotic/heavy metal among bacteria of Gangotri glacier (upper stretch) is comparatively less than that of lower and middle stretch. This situation of resistance pattern in bacteria at glacier is very alarming because most of the pilgrims take bath in rivers of lower and middle stretch and also villagers who are densely populated on the bank of the river use these water bodies as their drinking water source. Apart from R-factors bacteria may also harbour other plasmids such as those, which transmit enteropathogenicity among *E. coli*. Recombination between plasmids has been described by Watanabe (1963) and, thus the emergence and rapid spread of R-factors carrying genes which may convert *E. coli* into a drug resistant pathogen is a real possibility. An estimated 10ml accidental water intake implies that a person may ingest $2R^+$ coliforms during bathing (Wolf, 1972). In bathing water with a coliform

count of 10,000 per 100ml, or water polluted with sewage in which 20 percent of coliforms carry R factors the probable number of R^+ coliforms ingested during bathing increases to 20 (Grabow *et al.*, 1974). Drinking water may also contribute to the dissemination. A person drinks about 1400 ml of fluid daily (Guyton, 1971; Garbow *et al.*, 1974). Provided 100ml of this intake is from drinking water containing 10 coliforms of polluted origin per 100ml, $2R^+$ coliforms may be ingested each day. Such polluted water is directly or indirectly a common source of disease in man and animals. The health hazard imposed by R-factors is not restricted to drug resistance; they may enhance the infectivity and virulence of some pathogens. Thus observations make the statement of Paul Ehrlich prophetic when he said that 'resistance follows the antibiotic like a faithful shadow'. The abuse and misuse of antibiotics need to be curtailed. Strategies must take into account all factors influencing the prescription of antibiotic both in the field of medical and farm animals. Even with optimal antibiotic use antibacterial resistance will probably not decline quickly and existing resistances are unlikely to vanish (Levin *et al.*, 1998). Therefore, we must limit the diffusion of existing anti bacterial resistance in the population and avoid the emergence of new strains of resistant bacteria. In the present study microbial resistance in the runoff of the glacier, have revealed significant bacterial resistance in middle and lower stretch, therefore it is important to evaluate systematically the environmental conditions and cause of microbial resistance in the runoff of the glacier.

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Short communication

Sensitivity of newly released varieties of rice to herbicides.

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Abstract : A field experiment was carried out during Kharif 1999 at experimental farm of CSK HPKV Palampur to check the sensitivity of newly released varieties to herbicides in direct seeded puddled rice. Experiment was conducted in randomized block design with nine treatment combinations each replicated thrice. Treatments consisted of combinations of three-weed control methods viz., two hand weeding, butachlor 2.0 kg/ha and pretilachlor 0.8 kg/ha and three rice varieties RP-2421, HPR-957 and HPR-927. It can be concluded from the study that HPR-957 was sensitive to butachlor 2.0 kg/ha and pretilachlor 0.8 kg/ha herbicides.

Key words : Rice varieties, Herbicides, Sensitivity.

Introduction

In India, rice is grown as a staple food and accounts for about 31% of the total area under food grains and presently being grown on about 43 million hectares (Singhal, 1999). Rao (1983) reported 45% yield losses due to weeds, 30% due to insects, 20% by plant disease and 5% due to other pests. Rice (*Oryza sativa*), a major crop of Himachal Pradesh, suffers badly due to infestation of wide variety of grasses, sedges and broadleaf weeds. Hand weeding, is the most common and efficient practice but is slow, labor intensive and costly. Applications of pre-emergence herbicides have been recommended to control weeds in direct seeded upland rice. The information on the sensitivity of newly released varieties of rice is not available. In the present preliminary investigation, the sensitivity of newly released varieties to herbicides in direct seeded puddled rice has been reported.

Materials and Methods

A field experiment was conducted during Kharif 1999 at the Research farm of Department of Agronomy, CSK HPKV Krishi Vishvavidyalaya, Palampur. The soil of the experimental site was silty clay loam in texture, acidic in reaction, medium in available nitrogen and phosphorus and high in available potassium.

The experiment was laid out in a randomized block design with three replications. The sowing of the seeds of RP-2421, HPR-957 and HPR-927 varieties at 120 kg/ha was done on June 11, 1999 with recommended package of practices except weed control treatments. Experiment was conducted on randomized block design with nine-treatment combination each replicated thrice. Treatment consisted of combination of three weed control methods viz., two hand weeding at 20 DAS and 40 DAS, butachlor 2.0 kg/ha (3 DAS) and pretilachlor 0.8 kg/ha (Pre.) and three rice varieties RP-2421, HPR-957 and HPR-927. Herbicides were sprayed with Maruyama knapsack sprayer fitted with flat fan nozzle at spray volume of 600 litre/ha. The weed biomass was recorded at harvest. The data on crop for growth and grain yield were recorded.

Results and Discussion

The major weeds of the experimental field were *Echinochloa crusgalli*, *Panicum dichotomiflorum*, *Digitaria sanguinalis*, *Cyperus iria* and *Commelina benghalensis*. Rice variety RP-2421 and HPR-927 has showed no symptoms of toxicity due to pretilachlor 0.80 kg/ha application. Pretilachlor was observed to be best herbicide. Different varieties did not differ significantly in influencing the drymatter of weeds but variety HPR-957 being at par with RP-2421

produced significantly lower grain yield of rice. Both the herbicides were equally effective to increase grain yield of rice significantly by effective control of weeds. Interaction effect of

varieties and weed control methods reveal that inspite of equal drymatter of weeds due to both the herbicides, grain yield of variety HPR-957 was reduced significantly by butachlor 1.5 kg/ha over

Table - 1: Effect of weed control treatments on grain yield of rice and drymatter of the weeds

Treatments	Grain yield (kg/ha)	Drymatter of weeds (g/m ²)
Variety		
RP-2421	2125.9	8.9 (77.7)
HPR-957	1777.8	8.9 (78.4)
HPR-927	2194.5	8.3 (67.9)
CD 5%	370.9	NS
Weed control treatment		
2 Hand weeding	2314.8	3.2 (9.1)
Butachlor (2.0 kg/ha)	1724.1	11.2 (124.2)
Pretilachlor (0.8 kg/ha)	2059.3	11.7 (136.1)
CD 5%	370.9	1.1

Figures in parentheses indicate the original values.

Table - 2 : Interaction effect of rice varieties and herbicides on grain yield of rice and drymatter of weeds

Interaction	Grain yield (kg/ha)			Dry matter of weeds (g/m ²)		
	Butachlor	Pretilachlor	Hand weeding twice	Butachlor	Pretilachlor	Hand weeding twice
RP-2421	1733.34	2355.60	2288.90	11.6 (133.6)	11.9 (140.6)	3.1 (8.6)
HPR-957	1466.67	1644.45	2222.24	11.7 (135.9)	11.9 (140.6)	3.2 (9.2)
HPR-927	1972.23	2177.79	2433.35	10.3 (105.1)	11.4 (128.9)	3.3 (9.9)

Figures in parentheses indicate the original values.

	CD 5%
Grain yield	642.4
Dry matter of weeds	1.8

pretilachlor 0.8 kg/ha and hand weeding twice. It indicates that butachlor has phytotoxic effect to variety HPR-957. The grain yield of varieties RP-2421 was not affected significantly by different weed control treatments. The drymatter of weeds due to pretilachlor treatment was statistically equal in all the varieties but it has reduced the grain yield of variety HPR-957 significantly over variety RP-2421. It gives an indication that this variety also has phytotoxic reaction to pretilachlor.

It can be concluded from the study that HPR-957 was sensitive to butachlor and pretilachlor herbicides and needs intensive investigation.

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Review paper

Insect fauna associated with sugarcane plantations in Sri Lanka

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Abstract : A survey conducted over 13 years (1986-1999) in sugarcane plantations in Sri Lanka to identify insects associated with sugarcane recorded a total of 103 insect species comprising Coleoptera (31 spp.), Dictyoptera (2 spp.), Diptera (5 spp.), 12 Heteroptera (12 spp.), Homoptera (18 spp.), Hymenoptera (7 spp.), Isoptera (3 spp.), Lepidoptera (13 spp.), Orthoptera (9 spp.), and one species each of Thysanoptera, Neuroptera and Trichoptera. Among them were forty-six species of sugarcane pests. In addition, 27 species of natural enemies of sugarcane pests belonging to the orders Coleoptera, Diptera and Hymenoptera were identified. *Epiricania melanoleuca* (Fletcher) introduced into Sri Lanka from Pakistan in 1991 for the control of the sugarcane planthopper was also recorded. Five new pest species previously not recorded from sugarcane in Sri Lanka have been identified.

Key words : Insect diversity, Natural enemies, Sugarcane.

Introduction

Sugar production as an organized commercial industry in Sri Lanka began in the late 1950's even though growing of sugarcane in the country dates back to more than 100 years. Two small scale sugarcane plantations, initially established in Kantale and Hingurana (Eastern Province of Sri Lanka) in 1956 had been increased to 12936 ha by the year 1992 following the establishment of plantations in Sevanagala and Pelwatte (Uva province) (Fig 1). By 1999, an extent of 21, 000 ha was under sugarcane cultivation that included the private as well as commercial plantations.

New sugarcane varieties, which are hybrids of the genus *Saccharum*, meet 60% of the world's sugar demand (Stevenson, 1965). In Sri Lanka, majority of the commercial plantations are planted with the sugarcane variety Co 775. In addition, varieties SL 7103, SL 8306 and SLI 121 are also grown in few of the commercial plantations. Presence of graminious weeds in sugarcane plantations have contributed towards the population build up of many insect species. According to a recent survey, 36 species of grasses have been recorded from sugarcane plantations in Sri Lanka (Personal communication, W.R.G. Witharana). Of

them, 19 species have been recorded from the Sevanagala plantation alone (Witharana *et al.*, 1997).

The earliest published information on sugarcane insects of the world and their parasitoids was by Box (1953). In his list of sugarcane pests of the world the only record from Ceylon included in the list is *Pyrilla perpusilla* (referred to as *Pyrilla aberrans*). Fennah (1963) made a taxonomic study of *Pyrilla perpusilla*, the most serious planthopper pest of Ceylon and India. Rajendra (1979) who was the first to report on the sugarcane pests of Sri Lanka in two sugarcane plantations existed at that time recorded 110 insect species. Of them 60 species were considered as causing damage to sugarcane with varying degrees of importance. Six species of pests, *Pyrilla perpusilla singhalensis* Fennah, *Sesamia inferens* Walker Chilo sp., *Odontotermes* sp., *Aclerda takahashi* Kuwana and *Saccharicoccus sacchari* (Cockrell), were regarded as potentially important and few of them were assumed to have reached epidemic proportions at different times.

Insect pests of sugarcane have been reported from Afghanistan (Cotterrell, 1953), India (Butani, 1961; Parsana and Malawia, 1994), Nigeria (Wada, 1997), Pakistan (Chaudhry and Ansari, 1988) and several other parts of the world (Long and Hensley,

1972). However, there are no reports on the overall insect fauna associated with sugarcane from any part of the world.

This paper reports on the insects fauna associated with sugarcane including potentially important pests together with predators and parasitoids, from all the six sugarcane-growing areas of the country.

Materials and Methods

Insect surveys were carried out in all six sugarcane plantations; Hingurana (3400 ha), Kantale (2800 ha), Pelwatta (6600 ha), Sevanagala (3300 ha), Siyambalanduwa (3300 ha) and Uda Walawe (600 ha) between 1986-1999 (Fig 1). Sugarcane fields surveyed were at different ages, from planting

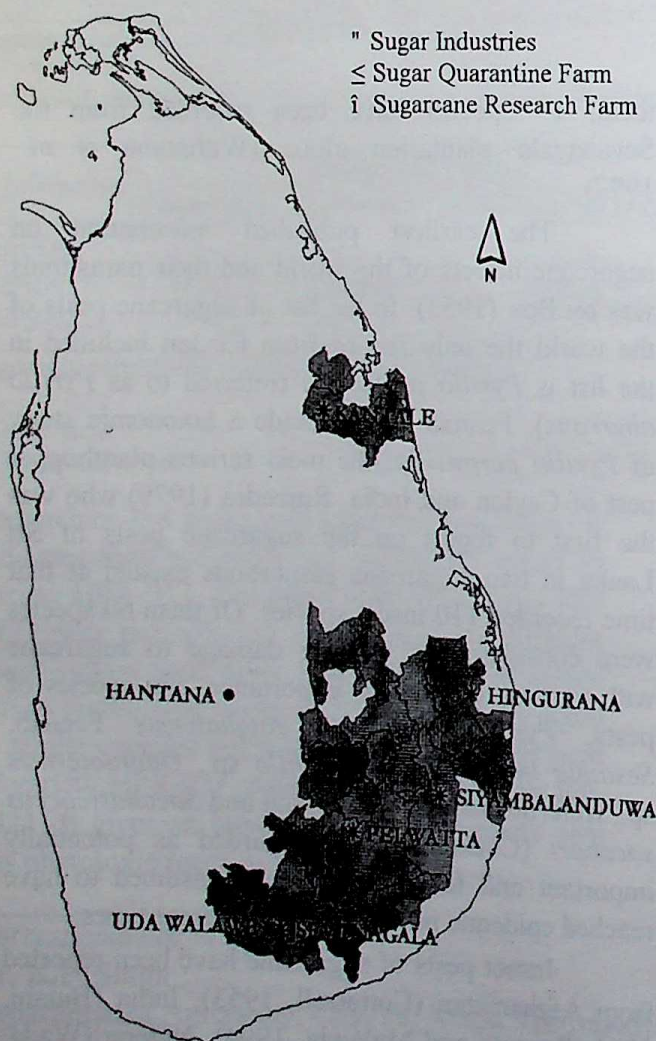


Fig. 1 : The location of the six sugarcane growing areas in Sri Lanka, surveyed during the study.

to harvest. Thus, insects were collected from the newly planted crop, ratoon crop, post harvest fields and the surrounding grasslands. The following methods were used to collect insects during the survey.

a) Collection of subterranean insects : Insects were manually collected after uprooting planted seed cuttings following visible signs of dead shoots. Termites collected from seed cuttings were preserved in 70% alcohol for identifications. The roots of mature sugarcane and grasses were similarly examined for insects. The soil around the roots of sugarcane and grasses upto a depth of 60 cm was also examined for subterranean insects.

b) Insects on vegetation : Insects on sugarcane and wild grasses were collected using an insect net and an aspirator. In addition, a portable light trap installed near the boundary of the plantations was used to collect night flying insects.

c) Stem boring and sap feeding insects of sugarcane and grasses : The larval stages feeding on internal stem tissues of sugarcane and grasses were collected by splitting open the damaged stems and removing the larvae. Scale insects and mealy bugs on the stems of sugarcane and grasses were also collected.

d) Insects in post harvest fields : Ants and termites present on stubbles of sugarcane in post harvest fields were collected manually. Larvae and adults of beetles associated with dead roots of sugarcane and grasses were also collected.

e) Collection of parasitoids of sugarcane pests : The stem borer larvae collected by splitting damaged stems were reared in the laboratory at room temperature ($30 \pm 2^\circ\text{C}$) until the emergence of parasitoids. The parasitoids of mealy bugs and scale insects were obtained by enclosing sections of infested stems in polythene bags until parasitoid emergence.

Identification of insects : The collected specimens were sent to the British Museum of Natural History, Natural Resources Institute (formerly TDRI and ODNARI), UK and the CAB International Institute of Entomology (Formerly CIE), UK for identification and return. Expert identified specimens are deposited

Insect diversity within sugarcane plantation.

in the Insect Collection of the Sugarcane Research Institute, Uda Walawe.

Results and Discussion

The 103 insect species collected from the sugarcane plantations in Sri Lanka over a period of 13 years are listed in Table 1. The parasitoids and predators of insects associated with sugarcane plantations are given separately in Table 2.

Insects belonging to 12 orders were associated with the sugarcane plantations. Of the 31 species of Coleoptera collected and identified from the sugarcane plantations, only five scarabaeid species, *Alisonotum* sp., *Alisonotum piceum*, *Anomala* sp., *Holotrichia serrata* and *Maladera* sp. were found to be pests attacking the roots of sugarcane. *Philodonata modesta* was found in sugarcane nurseries causing damage to seedlings.

Table - 1 : Insects associated with sugarcane plantations in Sri Lanka.

Species	Remarks on damage to sugarcane	Location
Coleoptera		
Carabidae		
<i>Craspedophorus elegans</i> (Dejean)	-	Kantale, Pelwatta
<i>Pseudognathaphanus punctilabris</i> (macleay)	-	Uda Walawe
Chrysomelidae		
<i>Chaetocnema basalis</i> (Baly)	Found on young sugarcane leaves	Uda Walawe
<i>Madurasia obscurella</i> Jacoby	-	Hingurana, Kantale
<i>Medythia suturalis</i> (Motschulsky)	-	Hingurana, Kantale
Curculionidae		
<i>Mylocherus curvicornis</i> (Fabricius)	-	Hingurana
Dytiscidae		
<i>Hyphydrus lyratus</i> Swartz	-	Hingurana
<i>Hydaticus</i> sp.	-	Hingurana, Kantale
<i>Hydrovatus</i> sp.	-	Hingurana, Kantale
<i>Neptosternus taprobanicus</i> Shrap	-	Hingurana, Kantale
Hespiidae		
<i>Philodonata modesta</i> Mots	Found in sugarcane nurseries causing damage to germinated seedlings	Sevanagala
Hydrophilidae		
<i>Hydrophilus caschmirensis</i> Redtenbacher	-	Pelwatta, Sevanagala
<i>Regimbartia attenuata</i> (Fabricius)	-	Pelwatta
<i>Sternolophus brachyacanthus</i> Regimbart	-	Sevanagala, Uda Walawe
<i>Sternolophus rufipes</i> (Fabricius)	-	Sevanagala
Nitidulidae		
<i>Haptoncus</i> sp.	-	Sevanagala
Noteridae		
<i>Canthydrus laetabilis</i> (Walker)	-	Sevanagala, Uda Walawe
Phalacridae		
<i>Phalacrus</i> sp.	These species are known to feed on smut fungi on sugarcane	Uda Walawe
Scarabaeidae		
<i>Alisonotum</i> sp.	Adult attacks germinated setts resulting death of the plant	Hingurana, Kantale, Pelwatta
<i>Alisonotum piceum</i> Fabricius	Adult attacks germinated setts resulting death of the plants seen in highlands specially during drought conditions	Hingurana, Kantale, Pelwatta
<i>Anomala</i> sp.	Found in soils near the root system of sugarcane	Siyambalanduwa,

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<i>Holotrichia serrata</i> Fabricius	Found in soils near the root system of sugarcane	Siyambalanduwa
<i>Onthophagus quadridentatus</i> (Fabricius)	-	
<i>Onthophagus cervus</i> (Fabricius)	-	
<i>Phyllognathus dionysius</i> (Fabricius)	Associated with decaying plant material	Pelwatta
<i>Rhyssalus inscitus</i> (Walker)	-	
<i>Maladera</i> sp.	Found in soils near the root system of sugarcane	Siyambalanduwa
Scirtidae	-	
<i>Ora picta</i> (Fabricius)	-	Siyambalanduwa
Staphylinidae	-	Siyambalanduwa
<i>Philonthus notabilis</i> Kraatz	-	
<i>Philonthus aeneipennis</i> Boheman	-	Siyambalanduwa
<i>Zyras hoplonotus</i> (Kraatz)	-	Uda Walawe
Dictyoptera		
Pycnoscelidae		
<i>Pycnoscelus surinamensis</i> (Linnaeus)	Wood roach species found in sugarcane plantations	Hingurana, Kantale, Sevanagala
Oxyhalopidae		
<i>Nauphoeta cinerea</i> (Olivier)	-	Kantale
Diptera		
Chloropidae		
<i>Scoliophthalmus memorialis</i> Kanmiya	Found in young shoots of sugarcane	Siyambalanduwa
Drosophilidae		
<i>Drosophila</i> sp.	This species is associated with moulds growing on Honeydew exuded by <i>Saccharicoccus sacchari</i>	Sevanagala
Ephydriidae		
<i>Paralimna hirticornis</i> Meijere	Found on grass of the leaf roller	Pelwatta, Uda Walawe
Otitidae		
<i>Physiphora clausa</i> Macquart	Found in decaying material near sugarcane fields	Pelwatta
Pipunculidae	-	Sevanagala
<i>Pipunculus</i> sp.		
Heteroptera		
Alydidae		
<i>Leptocoris oratorius</i> (Fabricius)	-	Uda Walawe
Belastomatidae		
<i>Sphaerodema rusticum</i> (Fabricius)	-	Pelwatta, Sevanagala
Cydidae		
<i>Geotomus</i> sp.	-	Pelwatta
<i>Macroscytus</i> sp.	-	Pelwatta
Lygaeidae		
<i>Horridipamera nietneri</i> (Dohrn)	-	Sevanagala, Uda Walawe
Pentatomidae		
<i>Carbula socia</i> Walker	-	Sevanagala
<i>Eusarcocoris ventralis</i> (Westwood)	-	Sevanagala
<i>Nezara viridula</i> (Linnaeus)	-	Sevanagala
<i>Plautia</i> sp.	-	Sevanagala
<i>Scotonophora coarctata</i> Fabricius	-	Sevanagala
<i>Stenozygum speciosum</i> Dallas	-	Sevanagala
Pyrrocoridae		
<i>Dysdercus</i> sp.	-	Uda Walawe
Homoptera		
Aclerdidae		
<i>Aclerda takahashii</i> Kuwana	Nymphs as well as adults suck sap from stalks. Live in colonies in staks.	All sugarcane growing areas

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Aleyrodidae <i>Neomaskellia bergii</i> (Signoret)	This species feed on Graminae but specially noted as a pest of sugarcane	Sevanagala, Uda Walawe
Aphidiade <i>Melanaspis sacchari</i> (Zehntner)	Small ant attended sap sucking aphid, occurs on sugarcane leaves.	Kantale, Siyambalanduwa
<i>Rhopalosiphum maidis</i> (Fitch)	Live on sugarcane leaves and grasses	Kantale
Cicadellidae <i>Cofana spectra</i> (Distant)	Common sap sucking species found on sugarcane and other grass species	Siyambalanduwa,
<i>Nephotettix bipunctatus</i> Fabricius	-	Sevanagala
Coccidae <i>Saccharolecanium kurgeri</i> (Zehntner)	Live in colonies in mature sugar cane	All sugarcane growing areas
Delphacidae <i>Perkinsiella saccharicida</i> Kirkaldy	Nymphs and adults suck sap from sugarcane leaves or tender parts of the stem	Hingurana, Kantale Siyambalanduwa
<i>Sogata</i> sp.	Nymphs and adults suck sap from sugarcane leaves	The first recorded of this species was in 1997 from the sugarcane quarantine farm Hantana. In 1998 this species was observed on wild sugarcane (<i>Erianthus</i>) in Peradeniya and Gampola areas.
<i>Tropidocephala signata</i> Distant	Nymphs and adults suck sap from sugarcane leaves or tender parts of the stem	Hingurana, Kantale Siyambalanduwa
<i>Tropidocephala saccharivorella</i> Mots	Nymphs and adults suck sap from sugarcane leaves or tender parts of the stem	Hingurana, Kantale Siyambalanduwa
Derbidae <i>Proutista moesta</i> (Westwood)	Adults rest on the ventral surface of sugarcane leaves	All sugarcane growing areas
Lophopidae <i>Lophos saccharicida</i> (Kirkaldy)	Nymphs and adults feed on sap of sugarcane leaves	Kantale, Siyambalanduwa, Pelwatta, Sevanagala
<i>Pyrilla perpusilla singhalensis</i> Fennah	This was a serious juice sucking pest of sugarcane in Sri Lanka until 1992. Adults and nymphs suck sap from sugarcane leaves causing damage to the yield.	All sugarcane growing areas
Pseudococcidae <i>Antonia graminis</i> (Maskell)	Colonize on sugarcane leaves and leaf sheath	Siyambalanduwa, Pelwatta
<i>Dysmicoccus brevipes</i> (Cockrell)	Sap sucking mealy bug species found on sugarcane leaves	Siyambalanduwa, Pelwatta
<i>Dysmicoccus boninsis</i> (Kuwana)	Sap feeding mealy bug species found on sugarcane leaves	The first record of this sugarcane leaves species was from the sugarcane quarantine farm, Hantana in 1993 All sugarcane growing areas
<i>Saccharicoccus sacchari</i> (Cockerell)	This species is commonly known as the pink sugarcane Mealy bug. It colonizes in between the leaf sheath and the stem and feed on sap heavy infestation can severely deplete the plant's sap. The species has been recorded on sorghum, rice and grass genera <i>Panicum</i> and <i>Imperata</i> too.	

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Hymenoptera		
Formicidae		
<i>Aenictus</i> sp.	Ants found on sugarcane stubbles after ratooning	Uda Walawe, Sevanagala
<i>Anoplolepis longipes</i> (Jerdon)	Ants found on sugarcane stubbles after ratooning	Uda Walawe, Sevanagala, Pelwatta
<i>Camponotus</i> sp.	Ants found on sugarcane stubbles after ratooning	Uda Walawe, Sevanagala
<i>Crematogaster</i> sp.	Ants associated with the sugarcane pink Mealy bug <i>Saccharicoccus sacchari</i>	Uda Walawe, Sevanagala
<i>Lophomyrmex quadrispinosus</i> (Jerdon)	Ants found on sugarcane stubbles after ratooning	Uda Walawe, Sevanagala, Pelawatte
<i>Meranoplus bicolor</i> (Guerin)	Ants found on sugarcane stubbles after ratooning	Uda Walawe, Sevanagala, Pelawatte
<i>Plagiolepis</i> sp.	Ants found on sugarcane stubbles after ratooning	Uda Walawe, Sevanagala
Isoptera		
Termitidae		
<i>Odontotermes ceylonicus</i> Wasmann	Feed on inner tissues of seed cane and mature cane. Attack the ratoon also after harvest. Higher levels of damage have been recorded during drought periods specially in highlands.	Pelwatta, Sevanagala, Uda Walawe
<i>Odontotermes horni</i> (Wasmann)	Feed on inner tissues of seed cane and mature cane. Attack the ratoon also after harvest. Higher levels of damage have been recorded during drought periods specially in highlands.	Pelwatta, Sevanagala, Uda Walawe
<i>Odontotermes redemanni</i> (Wasmann)	Feed on inner tissues of seed cane and mature cane. Attack the ratoon also after harvest. Higher levels of damage have been recorded during drought periods specially in highlands.	Pelwatta, Sevanagala, Uda Walawe
Lepidoptera		
Hesperiidae		
<i>Parnara narooa</i> Moore	The larva feeds on sugarcane leaves	Hingurana, Kantale
<i>Telicota augias</i> (Linnaeus)	The larva feeds on sugarcane leaves	Hingurana, Kantale
Limacodidae		
<i>Thosea aperiens</i> (Walker)	The larva feeds on sugarcane leaves	Hingurana, Kantale, Uda Walawe
Lymantriidae		
<i>Euproctis</i> sp.		Uda Walawe
Noctuidae		
<i>Agrotis biconica</i> (Kollar)	The larvae found in side the shoots of young sugarcane plants	Pelwatta
<i>Mithimna irregularis</i> (Walker)	The larva consumes young sugarcane leaves	Pelwatta
<i>Sesamia inferens</i> (Walker)	Spends early stages of the larval period in grasses and then bore into the shoot of young sugarcane plants causing death.	All sugarcane growing areas
<i>Spodoptera execta</i> Walker	The larva consumes the leaves of sugarcane	Hingurana, Kantale
Pyalidae		
<i>Ancylolomia indica</i> Felder and Rogenhofer	The larva feeds on young sugarcane leaves	Uda Walawe
<i>Canphalocrocis medinalis</i> (Guenée)	The larva feeds on young sugarcane leaves	Hingurana, Kantale
<i>Chilo sacchariphagus indicus</i> (Kapur)	The larva bore into the stem affecting the physiology, strength and the sugar content of the plant	All sugarcane growing areas
Sphinigidae		
<i>Leucophlebia lineata</i> Westwood	The larva feeds on young sugarcane leaves	Hingurana, Kantale
Tortricidae		
<i>Tetramoera schistaceana</i> (Snellen)	The larva feeds on young sugarcane leaves	Hingurana, Kantale

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Neuroptera		Kantale, Sevanagala
Chrysopidae		
<i>Mallada boninensis</i> (Okamoto)		
Orthoptera		
Acrididae		
<i>Acrotylus humbertianus</i> Saussure	Leaf feeders of young sugarcane plants	Hingurana, Kantale
<i>Atractomorpha crenulata</i> (Fabricius)	Leaf feeders of young sugarcane plants	Pelwatta
<i>Aularches miliaris</i> (Linnaeus)	Leaf feeders of sugarcane and grasses	Hingurana, Kantale
<i>Cyrtacanthacris tatarica</i> (Linnaeus)	Leaf feeders of sugarcane and grasses	Hingurana, Kantale
<i>Hieroglyphus banian</i> (Fabricius)	Leaf feeders of sugarcane and grasses	Hingurana, Kantale
<i>Morphacris fasciata</i> Thunberg	Leaf feeders on young sugarcane plants	Hingurana, Kantale
<i>Oxya hyla</i> Serv	Leaf feeders of sugarcane and grasses	Hingurana, Kantale
<i>Oxya japonica</i> (Thunberg)	Leaf feeders of sugarcane and grasses.	Hingurana, Kantale
<i>Patanga succincta</i> (Linnaeus)	Leaf feeders of sugarcane and grasses.	Hingurana, Kantale
Thysanoptera		
Thripidae		
<i>Fulmekiola serrata</i> Kobus	This species feeds on young sugarcane leaves	Siyamalanduwa
Trichoptera		
Hydropsychidae		
<i>Amphipsyche meridiana</i> Ulmer	-	Uda Walawe

The smut feeder *Phalacrus* which can be an indirect transmitter of the smut disease of sugarcane was also recorded from several plantations.

Of the 18 species of Homoptera recorded, the acleridid, *Aclerda takahashii* and the coccid *Saccharolecanium kurgeri* and the whitefly *Neomaskellia bergii* are important pests of sugarcane. The aphid *Melanapis sacchari* which has been recorded as a vector of several viral diseases elsewhere was also recorded from sugarcane in several plantations. Of the delphacids, *Perkinsiella saccharicida*, *Tropidicephala signata* and *Tropidocephala saccharivorella* were the leaf hoppers on sugarcane. *Perkinsiella saccharicida* have been recorded as a vector of the Fiji disease of sugarcane in other sugarcane growing countries. *Sogata* sp. has not been previously recorded from Sri Lanka and it was collected from the sugarcane quarantine farm at Hantane. The pink mealy bug *Saccharicoccus sacchari* which is a pest of sugarcane was also recorded from all the plantations. The lophopid *Pyrilla perpusilla singhalensis* which had been a serious pest of sugarcane in Sri Lanka until 1992 was recorded from all sugarcane growing areas.

The three species of termites; *Odontotermes ceylonicus*, *Odontotermes horni* and *Odontotermes redemanni* which severely affect the rainfed

sugarcane plantations in the dry zone were also among the insect species identified.

Of the Lepidopterans, the noctuid *Sesamia inferens* which is a well known pest of sugarcane and rice was recorded from all six sugarcane plantations surveyed. The Pyralid stalk borer *Chilo sacchariphagus indicus* is an important pest which affects the physiology, stalk strength and the sugar content of sugarcane by making galleries in the sugarcane stalks.

The insect species found on both sugarcane and grasses in the surveyed plantations are the Orthopteran acridids *Acrotylus humbertianus*, *Atractomorpha crenulata*, *Aularches miliaris*, *Cyrtacanthacris tatarica*, *Hieroglyphus banian*, *Morphacris fasciata*, *Oxya hyla* and *Patanga succincta* and the Heteropteran cicadellid *Cofana spectra*, alydid *Leptocoris oratorius*, lygaeid *Horridipamera nietneri*, pentatomids *Carbula socia*, *Eusarcoris ventralis*, *Nezara viridula*, *Scotonophora coarctata* and the pyrrhocorid *Dysdercus* sp.

The natural enemies of sugarcane pests belonging to the Orders Coleoptera, Diptera, Hymenoptera and Lepidoptera were present (Table 2). Of the natural enemies recorded, six species of coccinellid predators and twenty species of parasitoids of sugarcane pests which can be used in

biological control programmes were also identified during the survey. The Lepidopteran epipyropid, ectoparasitoid *Epiricania melanoleuca* was introduced into Sri Lanka in 1991 from Pakistan for

biological control of *Pyrilla perpusilla*. *Epiricania melanoleuca* was recorded from all the six plantations, thus indicating its establishment in Sri Lanka.

Table - 2 : Natural enemies of insects associated with sugarcane plantations in Sri Lanka.

Species	Host
Coleoptera	
Coccinellidae	
<i>Brumoides suturalis</i> (Fabricius)	Feed on the eggs and nymphs of <i>P. perpusilla</i>
<i>Harmonia octomaculata</i> (Fabricius)	Predatory species on Aphids
<i>Micraspis discolor</i> (Fabricius)	Predatory species on Aphids and nymphs of <i>P. perpusilla</i>
<i>Micraspis allardi</i> (Mulsant)	Predatory species on Aphids and nymphs of <i>P. perpusilla</i>
<i>Ortalia</i> sp.	Predatory species on Aphids
<i>Platynaspis</i> sp.	Predatory species on Aphids
Diptera	
Drosophilidae	
<i>Caxoxenus perspicax</i> Knab	The larva of this species develops on <i>Saccharicoccus sacchari</i> .
Tachinidae	
<i>Alsomyia Anomala</i> Villeneuve	Parasitic on sugarcane shoot borer <i>Sesamia inferens</i> and <i>Mythimna irregularis</i> larvae
Hymenoptera	
Aphelinidae	
<i>Promuscia infasciiventris</i> Girault	Primary parasitoid of the pink mealy bug <i>Saccharicoccus sacchari</i> and a hyper parasitoid of <i>Anagyrus saccharicola</i>
Braconidae	
<i>Apanteles javensis</i> Rohwer	Parasitoids of the sugarcane leaf roller
<i>Cotesia flavipes</i> Cameron	Larval parasitoid of the stem borer <i>Chilo Sacchariphagus indicus</i>
<i>Aphanogmus fijiensis</i> Ferriere	A hyper parasitoid of <i>Cotesia flavipes</i>
Ceraphronidae	
<i>Aphanogmus fijiensis</i> Ferriere	A hyper parasitoid of <i>Cotesia flavipes</i>
Encyrtidae	
<i>Anagyrus saccharicola</i> Timberlake	Parasitoid of the pink mealy bug <i>Saccharicoccus sacchari</i> (Noyes and Hayat, 1994).
<i>Astymachus japonicus</i> Howard	Parasitic on <i>Melanaspis glomerata</i> and <i>Saccharicoccus sacchari</i> .
<i>Cheiloneurus pyrillae</i> Mani	Parasitic on <i>P. perpusilla</i> eggs.
<i>Mayridia</i> sp.	Parasitoid of the sugarcane scale insect <i>Aclerda takahashi</i> (Jadhava <i>et al.</i> , 1991)
<i>Neastymachus auraticorpus</i> Girault	Parasitoid of the sugarcane scale insect <i>Aclerda takahashi</i> (Jadhava <i>et al.</i> , 1991)
Eulophidae	
<i>Parachrysocharis javensis</i> Girault	Parasitoid of <i>P. perpusilla</i> eggs (Kumarasinghe and Wratten, 1996)
Eupelmidae	
<i>Anaustaus</i> sp.	Parasitoids of wood roach oothecae
Ichneumonidae	
<i>Brachycyrtus</i> sp.	A lacewing parasitoid
Ryiniidae	
<i>Richardsidryinus pyrillae</i> (Kieffer)	Primary ectoparasitoid of <i>P. perpusilla</i> (Kumarasinghe and Wratten, 1996)
Scelionidae	
<i>Telenomus dignus</i> Gahan	Primary egg parasitoid of the stem borer <i>Chilo Sacchariphagus indicus</i>
<i>Baryconus graveleyi</i> (Mani)	Recorded as parasitoids of grass hopper eggs (Mani, 1939; 1942)

.....continued on to next page

Trichogrammatidae
Trichogramma Chilonis Ishii
 Lepidoptera
 Epipyropidae
Epiricania melanoleuca (Fletcher)

Egg parasitoid of the stem borer *Chilo sacchariphagus indicus*

Larvae of this moth are ectoparasitic on nymphal and adult stages of the planthopper *Pyrilla perpusilla singhalensis*. This parasitoid was introduced in 1991 from Pakistan to Sri Lanka by the Sugarcane Research Institute in order to control *P. perpusilla*. The family has been reviewed by Krampl and Dlabola (1983).

It is important to note that the lophopid *Lophos saccharicida* recorded for the first time by Rajendra (1979) continues. The present survey reveals that the majority of the sugarcane pests such as *Saccharicoccus sacchari*, *Melanapis sacchari*, *Aclerda takahashii*, *Perkinsiella saccharicida*, *Tropidocephala saccharivorella*, *Tropidicephala signata*, *Proutista moesta*, *Sesamia inferens*, and *Chilo sacchariphagus indicus* recorded by Rajendra (1979) are still present in all the plantations surveyed. In addition, several sugarcane pests such as; the delphacid *Sogata* sp., coccid *Saccharolecanium kurgeri*, and pseudococcids *Antonia graminis*, *Dysmicoccus brevipes* and *Dysmicoccus boninsis* were recorded for the first time in Sri Lanka during the present survey.

The survey further confirmed that certain economically important pests of sugarcane recorded from other countries such as *Emmalocera depressella* Swinh (Lepidoptera : Pyralidae) from Afganistan, *Aleurolobus barodensis* Mask (Homoptera : Aleyrodidae), *Scirpophaga nivella* (F.) (Lepidoptera : Pyralidae) from India, *Eldana saccharina* Walker (Lepidoptera : Pyralidae) from Nigeria, *Bissetia steniellus* (Hampson) (Lepidoptera : Crambinae) from Pakistan and *Saccharosydne saccharivora* (Westwood) (Hemiptera : Delphacidae) from Jamaica, Trinidad and Venezuela are not present in Sri Lanka.

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Pre-administration of β -carotene protects tissue glutathione and lipid peroxidation status following exposure to gamma radiation

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Abstract : The present study has been aimed to investigate the protective effect of β -carotene against radiation-induced oxidative stress in mice tissues using lipid peroxidation and glutathione (GSH) as end points. Fourteen days oral priming administration of β -carotene (35 mg/kg body weight) followed by an acute dose of gamma radiation (5 Gy) inhibited the augmented level of thiobarbituric acid reactive substance (TBARS) and a statistically significant protection against GSH depletion. Results evaluated from this study clearly indicate the antioxidative property of β -carotene against gamma radiation, which is suggestive of free radical scavenging and singlet oxygen quenching.

Key words : Lipid peroxidation, β -carotene, Free radicals, Radiation.

Introduction

Modification of radiation effects through chemical protection of normal tissue has been practiced over the past 50 years. Some vitamins and provitamins display antimutagenic and anticarcinogenic properties against radiation (Ni and Pei, 1997; Sies and Stahl, 1995; Benova, 1992). Although it is not easy to define a compound as an anticarcinogen, but β -carotene, a well-known antioxidant, plays an important role in reducing the mutagenicity of many chemicals (Mukerjee *et al.*, 1991; Salvadori *et al.*, 1994). It has been observed that β -carotene is a potent free radical scavenger, singlet oxygen quencher and lipid antioxidants (Gey, 1994; Blot *et al.*, 1995). The observational epidemiological studies continue to accumulate impressive evidence that antioxidant vitamins and β -carotene (Kohlmeir and Hastings, 1995) rich food have an important role in the prevention of cardiovascular diseases, as well as in cancer (Byers and Guerrero, 1995; Van Poppel and Goldbohm, 1995). It has, therefore, been recommended that β -carotene be used for protection against certain types of human cancer and photosensitized oxidative damage (Bertram and Bortkiewicz, 1995; Albanes *et al.*, 1995) because free radicals and related reactive species may play some role in their pathology. In our previous study, it has been reported that β -carotene prevents the radiation-induced lipid peroxidation in

mice liver and spleen (Ramesh *et al.*, 1997). The present study is hence an attempt to document the radioprotective effect of β -carotene in various tissues of mice.

Materials and Methods

Swiss albino mice were selected from an inbred colony and maintained on standard mice feed (Hindustan Lever Ltd., New Delhi) and water *ad libitum*. β -carotene, thiobarbituric acid and glutathione were purchased from Sigma, USA. All other chemicals used were of analytical grade.

In our previous study the dose dependent effectiveness of β -carotene against lethal gamma radiation, it has been found that β -carotene has its higher efficacy at 30 mg dose level amongst 20, 30, 50 and 70 mg regimen. Other doses have been either less effective or toxic (Bhatia *et al.*, 2001). Therefore, present study has been carried out using a single dose level of β -carotene i.e. 30 mg/kg body weight. Swiss albino mice of 6-8 week age group were selected from an inbred colony and divided into four groups : The first group served as normal (did not receive any treatment). Second group was administered β -carotene (30 mg/kg body weight) orally for two weeks. Third group (control) was exposed to 5 Gy gamma radiations. Fourth group (experimental) was administered β -carotene (30 mg/kg body weight) orally for two weeks and then

exposed to 5 Gy gamma radiations. The animals were sacrificed 24 hrs post exposure and various organs viz., brain, liver, spleen, kidney, lungs and testis were removed for the estimation of lipid peroxidation and glutathione. Lipid peroxidation as reflected by thiobarbituric acid reactive substances (TBARS) or malondialdehyde (MDA) equivalent content was estimated by the method of Ohkawa *et al* (1979) using tetramethoxypropane (malondialdehyde) as the standard. GSH was measured as described by Ellman and Archs (1959). In this widely used spectrophotometric GSSG recycling assay, the rate of formation of 2-nitro-5-thiobenzoic acid is measured at 412 nm in a system containing 5,5-dithiobis- (2-nitrobenzoic acid) (DTNB) reagent. The values are expressed as mean \pm S.E.M of six animals. The difference between various groups was analyzed by Student's t-test using $P < 0.001$ as highest level of significance.

Results and Discussion

A profound increase in the level of lipid peroxidation was observed after exposure to radiation (Table-1). The value of TBARS equivalents were augmented as per the following order :

Spleen > Liver > Kidney > Brain > Lungs > Testes

Radiation induced augmentation in the level of TBARS significantly declined in β -carotene treated irradiated group. Percentage protection evaluated in the terms of TBARS equivalents was in the following order :

Testes (46.19%) > Spleen (42.21%) > Lungs (40.44%) > Brain (33.52%) > Kidney (21.61%) > Liver (18.99%).

The radiation-induced depletion in the level of GSH (Table-2) was also ameliorated significantly ($p < 0.001$) in β -carotene treated irradiated group. As it is clear from the results that, value of GSH in β -carotene treated irradiated group is near the normal value.

β -carotene (only) treated groups show considerably lower value of TBARS and higher value of GSH in comparison to the normal. However, statistically it is not significant in the case of spleen, liver and kidney. Present findings corroborate the idea that depletion of glutathione results in enhanced lipid peroxidation (Anderstam *et al.*, 1992) and excessive lipid peroxidation can cause increased glutathione consumption (Comporti, 1987).

In present study the reduction in the augmented level of TBARS equivalents and elevation in GSH level in the β -carotene-treated animals suggests that β -carotene may scavenge the free radicals formed during oxidative stress. GSH with its sulphhydryl group functions in the maintenance of sulphhydryl groups of other molecules (especially proteins), as a catalyst for disulfide exchange reactions, and in the detoxification of foreign compounds, hydrogen peroxide and free radicals. When GSH acts as a reducing agent, its SH becomes oxidized and forms a disulfide link with other

Table - 1 : The amount of TBARS (n mol/g tissue) in mice tissues 24 hrs post treatment (with and without β -carotene). Values \pm S.E.M.

Tissues	Normal	β -carotene	Control	Experimental	Protection
Brain	140.2 \pm 1.3 ^{a*} (100%)	148.4 \pm 1.2 ^{b#} (105.85%)	193.4 \pm 1.2 (137.94%)	146.4 \pm 1.3 ^{c*} (104.42%)	33.52%
Spleen	339.3 \pm 2.4 ^{a*} (100%)	347.9 \pm 2.5 ^{b*} (102.53%)	492.5 \pm 2.8 (145.12%)	349.3 \pm 2.7 ^{c*} (102.94%)	42.21%
Liver	315.4 \pm 2.1 ^{a*} (100%)	327.4 \pm 2.3 ^{a*} (103.80%)	383.2 \pm 2.5 (121.17%)	322.3 \pm 2.7 ^{c*} (102.18%)	18.99%
Testes	94.6 \pm 0.9 ^{a*} (100%)	105.6 \pm 0.7 ^{b#} (111.63%)	141.0 \pm 0.8 (149.04%)	97.3 \pm 1.3 ^{c*} (102.85%)	46.19%
Lung	122.3 \pm 1.3 ^{a*} (100%)	131.0 \pm 1.5 ^{b#} (107.11%)	183.5 \pm 1.2 (150.04%)	134.1 \pm 1.3 ^{c*} (109.6%)	40.44%
Kidney	214.7 \pm 2.1 ^{a*} (100%)	222.6 \pm 2.5 ^{b*} (103.67%)	274.3 \pm 2.3 (127.75%)	227.9 \pm 2.4 ^{c*} (106.14%)	21.61%

a = Statistical difference with experimental; b = Statistical difference with normal and c = Statistical difference with control
 * = $P < 0.001$; # = $P < 0.01$ and \diamond = non-significant. Statistical significance between various groups is evaluated by student's t test.
 Where, Normal = non-treated mice, β -carotene = β -carotene treated, Control = irradiated mice; Experimental = β -carotene treated + irradiated mice.

Pre-administration effect of β -carotene in various tissues of mice.Table - 2 : The amount of glutathione (nmol/g) in mice tissue 24 hrs post treatment (with and without β -carotene). Values \pm S.E.M.

Tissues	Normal	β -carotene	Control	Experimental
Brain	1.882 \pm 0.078 ^{a*}	2.103 \pm 0.078 ^{b#}	1.045 \pm 0.028	1.692 \pm 0.099 ^{c*}
Spleen	3.155 \pm 0.008 ^{a*}	3.172 \pm 0.008 ^{b*}	1.357 \pm 0.076	3.184 \pm 0.008 ^{c*}
Liver	7.673 \pm 0.314 ^{a*}	7.687 \pm 0.314 ^{b*}	4.312 \pm 0.412	7.501 \pm 0.314 ^{c*}
Testes	1.033 \pm 0.041 ^{a*}	1.321 \pm 0.041 ^{b#}	0.433 \pm 0.041	1.006 \pm 0.041 ^{c*}
Lung	1.813 \pm 0.156	2.021 \pm 0.156 ^{b#}	0.609 \pm 0.032	1.722 \pm 0.156 ^{c*}
Kidney	2.613 \pm 0.83 ^{a*}	2.701 \pm 0.83 ^{b*}	1.732 \pm 0.032	2.524 \pm 0.83 ^{c*}

a = Statistical difference with experimental; b = Statistical difference with normal and c = Statistical difference with control
 * = $P < 0.001$; # = $P < 0.01$ and \diamond = non-significant. Statistical significance between various groups is evaluated by student's t test.
 Where, Normal = non-treated mice, β -carotene = β -carotene treated, Control = irradiated mice; Experimental = β -carotene treated + irradiated mice.

molecules of GSH. Its high redox potential renders GSH both a potent antioxidant *per se* and a convenient cofactor for enzymatic reactions that require readily available electron pairs, the so-called "reducing equivalents" (Kehrer and Lund, 1994).

The antioxidative mechanism of β -carotene has been suggested to be singlet oxygen quenching, free radical scavenging and chain breaking during lipid peroxidation (Esterbauer, 1985). If carotenoids are to produce an antiradiation effect, it must be absorbed by the body and available in the tissue exposed to radiations. Adipose tissue, liver and plasma of humans were found to be the major pools of β -carotene (Gerester, 1993). β -carotene normally predominates among the most important carotenoids and is absorbed in the intestine. For humans one-sixth (on a weight basis) of the dietary β -carotene is estimated to be absorbed and converted to retinol whereas for other provitamin A carotenoids, about one-twelfth is absorbed and converted to retinol (Goodman *et al.*, 1966). The finding that β -carotene reduces the amount of TBARS or MDA equivalents in mice tissues when given before radiation exposure makes it a potential preventive agent against lipid peroxidation that can be induced by exposure to radiation. Nevertheless, more studies should be carried out to clarify the best dose and treatment schedules for the use of β -carotene as a radioprotective agent.

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Influence of pH, salt concentration and temperature on the growth of *Aeromonas hydrophila*

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Abstract : The influence of environmental factors on the growth of *Aeromonas hydrophila* was investigated. Four isolates (AH 37, AH 79, AH 86 and AH 100) were exposed to various environmental factors such as pH, salt concentration and temperature in laboratory condition. All the four isolates showed more or less similar growth at pH 7.0, 8.0 and 9.0 at 30°C and 5°C. At pH 5.0, 6.0 and 10.0, the log number of cells was found to be lesser than that of pH 7.0, 8.0 and 9.0 at both 30°C and 5°C. The results of the influence of salt concentration on the growth of *Aeromonas hydrophila* revealed that NaCl concentration of 0.5%, 1.0% and 2.0% favored the growth of this organism at both 30°C and 5°C. Increase in the salt concentration resulted in the growth of the decrease of this organism. Three percentages and 4% salt concentration moderately supported the growth of the organisms in the medium whereas at 5.0% NaCl concentration, there was no growth. Moderate growth of *A. hydrophila* at 5°C is an interesting observation. The ability to grow at salt concentration between 0.5%, 4.0% under acidic and alkaline conditions pose a problem in the preservation of seafoods. These criteria may account for modified preservation techniques.

Key words : *Aeromonas hydrophila*, pH, Temperature, Sodium chloride.

Introduction

Bacteria belonging to the genus *Aeromonas* are widely distributed in nature but are most prevalent in water. They have been isolated from a variety of aquatic habitats (Hazen *et al.*, 1978) and are considered normal microbiota of both the intestinal tract and external surfaces of freshwater fish (Cahill, 1990). Considering the ubiquitous nature of *Aeromonas* spp., it is not surprising that large variations are found between individual isolates in terms of temperature tolerance as well as other factors affecting growth and survival. Most growth kinetic studies are based on the behavior of one or very few strains. Predictive models based on these studies may therefore not cover the behavior of all *Aeromonas* spp., in most cases, however, clinical strains are used which seem to be relevant from a food infection point of view. If aeromonads reach high numbers in foods, it is theoretically possible that food poisoning could result not only from colonization (anherent exotoxin-producing strain), but also by intoxication (toxin elaborated into foods) following ingestion of contaminated foods that have

been under refrigeration, and / or have suffered temperature abuse, particularly if the foods are consumed with titles or no further cooking.

Factors influencing the lag time have been listed as : (i) change in nutrition, (ii) change in physical environment, (iii) presence of an inhibitor, (iv) spore germination in case of spore formers, and (v) the state of the inoculum culture (Pirt, 1975). Probably the single most important factor influencing lag time is incubation temperature. However, the lag time at a given temperature is also likely to be affected by the temperature at which the bacteria were grown, as well as other factors.

Not only are the rates of growth of microorganisms affected by pH, but also are the rates of survival during storage, heating, drying, and other forms of processing. In addition, the initial pH may be suitable, but because of a competitive flora or growth of the organism itself, the pH may become unfavourable. Conversely, the initial pH may be restrictive, but the growth of a limited number of microorganisms. The ability of low pH to restrict microbial growth has been deliberately employed

since the earliest times in the preservation of foods with acetic and lactic acids.

Palumbo (1988) investigated the combined effects of pH and sodium chloride, another important factor, which can influence the behaviour of *A. hydrophila*, on growth of the organism in ground pork at 5°C. *A. hydrophila* was sensitive to pH below 6.0 when initial pH in the pork was decreased from 6.1 to 5.9. The presence of 3% sodium chloride in vacuum-packaged ground pork suppressed the growth of *A. hydrophila* over 22 day storage period at 5°C (Palumbo, 1988). The survival of *A. hydrophila* in different environmental conditions has been reported in many parts of the world. These organisms are capable of growing in a diverse spectrum of pH, temperature and salt (NaCl) concentrations. Hence, the present investigation was conducted to monitor the influence of environmental factors such as pH, temperature and salt (NaCl) concentrations on the growth of *A. hydrophila*.

Materials and Methods

Bacterial cultures : Four strains of *A. hydrophila* (AH-37, AH-79, AH-86 and AH-100), which were isolated from body surface, gill, and intestine of fish and body surface of prawn respectively were selected. The influence of environmental factors such as pH, salinity and temperature on the growth of these strains was tested.

Test medium : Brain Heart Infusion Broth (BHIB, HIMEDIA) was used as the test medium. To adjust various pH levels and salt concentrations, it was modified by the addition of 1N. HCl or 1N NaOH and NaCl respectively.

Preparation of inoculum : *A. hydrophila* cells were grown in BHIB for 18 hours at 37°C. The cells were concentrated by centrifuging at 3000 rpm for 15 minutes and washed three times with 0.85% sterile NaCl solution. After the final wash, the cells were inoculated at an initial concentration of 4×10^6 cfu/ml to different test solutions.

Variables : The effect of temperature, percentage of salt (NaCl) and pH on the growth of selected strains was studied. The pH values selected were 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. The NaCl levels studied were

: 0.5%, 1.0%, 2.0%, 4.0% and 5.0%. The effects of salt and pH were studied at 30°C and 5°C.

Bacterial enumeration : Bacterial enumeration was done after 24, 48 and 72 hours of incubation. Bacterial quantification was made by surface overlaying procedure (Ray and Speck, 1973) on nutrient agar medium and the number of cells was expressed as colony forming units (cfu) per ml.

Results and Discussion

Influence of pH on the growth of *A. hydrophila* : All the four selected strains AH-37, AH-79, AH-86 and AH-100) showed more or less similar growth at various pH values at 30°C and at 5°C. *A. hydrophila* isolated from body surface of the finfish (AH-37) grew well at pH values 7.0, 8.0 and 9.0. After 72 h of incubation at 30°C, the bacterial population reached log 12.44, 12.79 and 12.20 number of cells respectively. Acidic pH and alkaline pH negatively influenced the growth of this organism. At pH 5.0 and 6.0, the bacterial populations were found to be 9.51 and 11.20 log number of cells after 72 h at 30°C, and at pH 10.0 also more or less similar growth (9.27 log number of cells) was detected (Fig. 1a) Similar variations in growth were noticed for other strains also (Fig. 1b-d).

At 5°C the growth of *A. hydrophila* increased from pH values 5-9 and maximum growth was noticed at pH 9.0. The growth was found to be 9.55, 9.32 and 9.63 log number of cells in the pH 7.0, 8.0 and 9.0 respectively. There was a reduction in the growth of *A. hydrophila* in acidic pH (5.0 and 6.0) and alkaline pH (10.0) and resulted in 8.39, 8.11 and 8.07 log number of cells respectively. In all the pH, there was a reduction in the number of cells after 24 h at 5°C. Further, the organism stabilized and gradually showed growth and yielded above 8.0 log number of cells after 72 h (Fig 1 e). Similar trend of growth was noticed for other strains also (Fig. 1f-h).

Influence of salt concentration on the growth of *A. hydrophila* : The influence of various salt concentrations on the growth of four strains of *A. hydrophila* (AH-37, AH-79, AH-86 and AH-100) was recorded at 30°C and 5°C. The strains yielded a considerable difference in the log number of cells

Influence of environmental factors on *Aeromonas hydrophila*.

when grown in various salt concentrations. Lower NaCl concentrations (0.5-2%) favored the growth of *A. hydrophila* while the concentrations (3-5%) were not supporting the growth of this organism at 3°C (Fig. 2a-d).

The strain AH-100 yielded log 12.2 number of cells at 0.5% of salt and exhibited 11.6 log number of cells in 1.0% salt concentration at 72 h. The growth at 2.0% salt concentration was found to be 10.2 log number of cells. Other strains such as AH-86, AH-79 and AH-37 showed 11.6, 10.7 and 10.63 log number of cells at 0.5% salt concentration respectively after 72 h. In 1.0% salt concentration,

AH-86 AH-37 and AH-79 the population increased to 11.5, 10.41, and 10.4 log number of cells respectively after 72 h. At 2.0% salt concentration, the growth of the selected strains were observed as 10.5, 10.4 and 10.36 log number of cells for AH-86, AH-79 and AH-37 respectively after 72 h.

Increase of the salt concentration, resulted in the decrease of the growth of the organism. A salt concentration of 3.0% also moderately influenced the growth of the strain and the log number of cells was found to be 10.11 (AH-37), 10.1 (AH-79), 9.4 (AH-86) and 9.3 (AH-100) respectively. At 4% salt concentration, the cell numbers (AH-37 and AH-79)

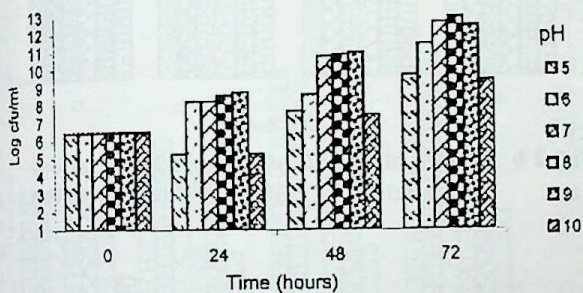


Fig. 1 a : Growth of *A. hydrophila* (AH-37) in various pH at 30°C

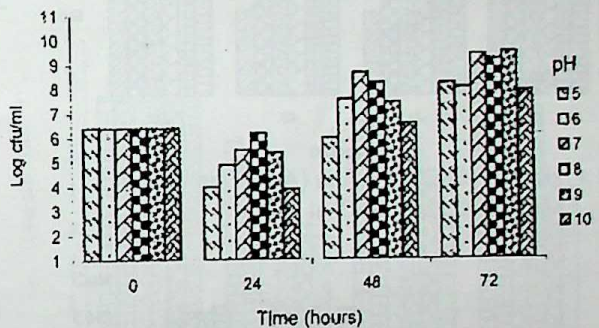


Fig. 1 b : Growth of *A. hydrophila* (AH-37) in various pH at 5°C

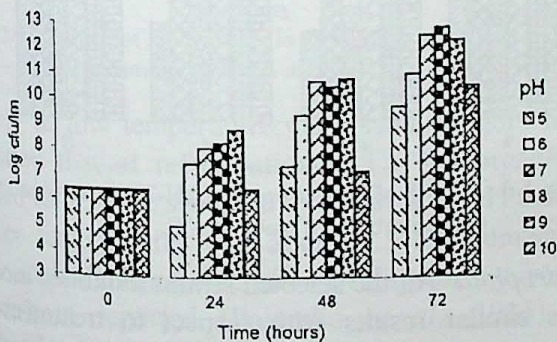


Fig. 1 c : Growth of *A. hydrophila* (AH-79) in various pH at 30°C

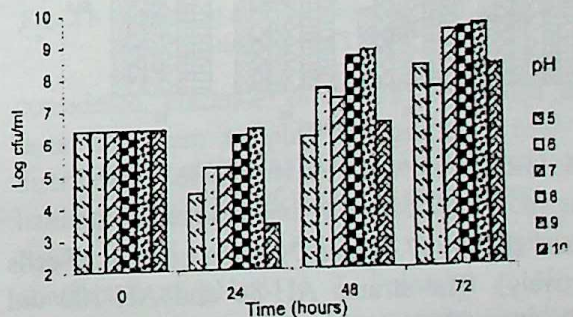


Fig. 1 d : Growth of *A. hydrophila* (AH-79) in various pH at 5°C

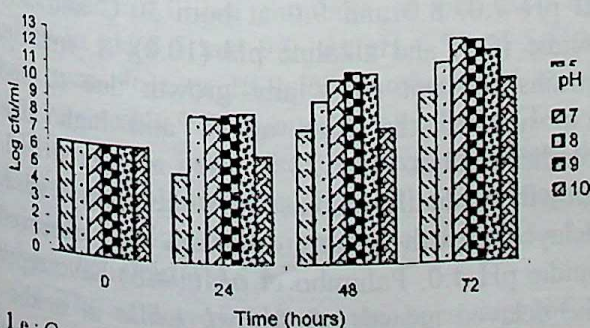


Fig. 1 e : Growth of *A. hydrophila* (AH-86) in various pH at 30°C

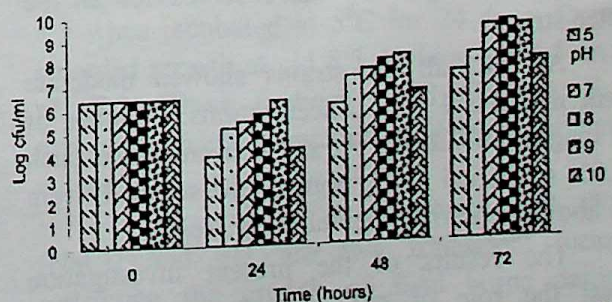


Fig. 1 f : Growth of *A. hydrophila* (AH-86) in various pH at 5°C

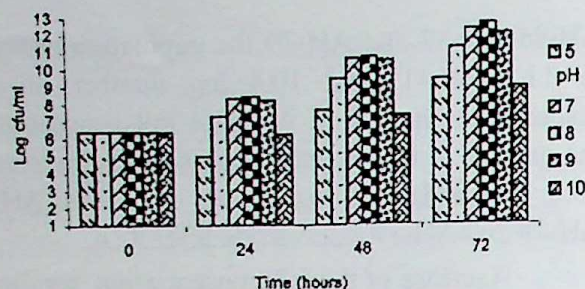


Fig. 1 g : Growth of *A. hydrophila* (AH-100) in various pH at 30°C.

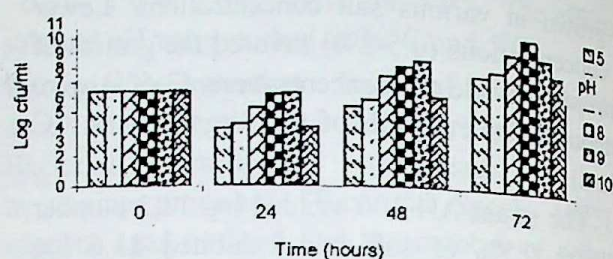


Fig. 1 h : Growth of *A. hydrophila* (AH-100) in various pH at 5°C.

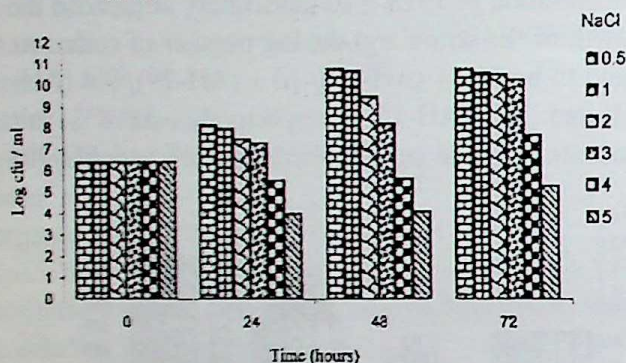


Fig. 2 a : Growth of *A. hydrophila* (AH-37) in various concentrations of NaCl at 30°C.

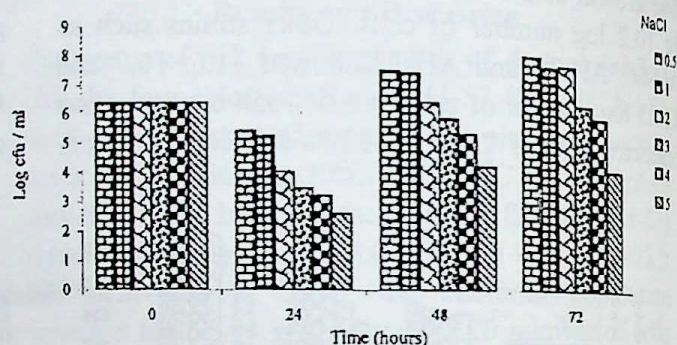


Fig. 2 b : Growth of *A. hydrophila* (AH-37) in various concentrations of NaCl at 5°C temperature.

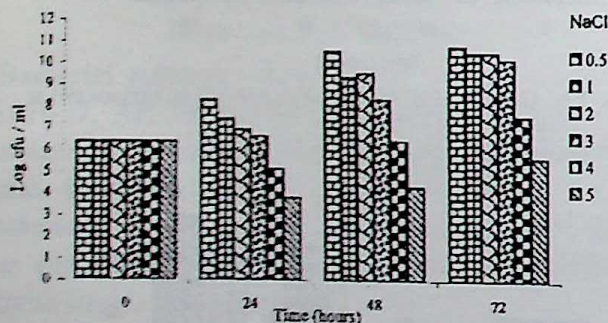


Fig. 2 c : Growth of *A. hydrophila* (AH-79) in various concentrations of NaCl at 30°C.

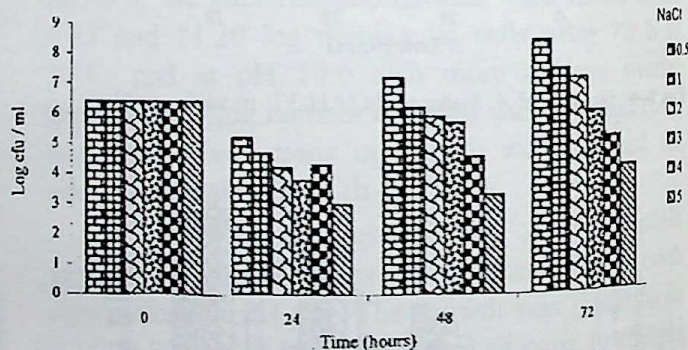


Fig. 2 d : Growth of *A. hydrophila* (AH-79) in various concentrations of NaCl at 5°C.

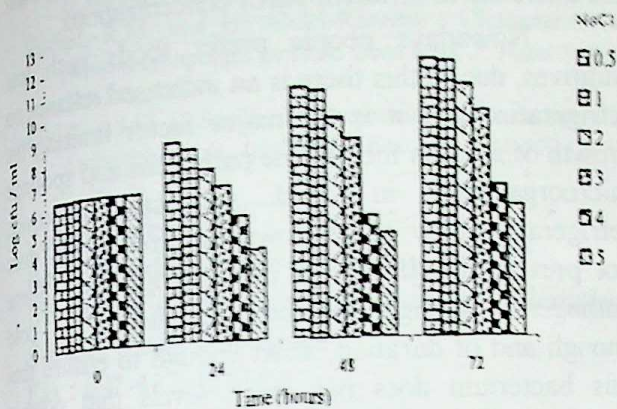
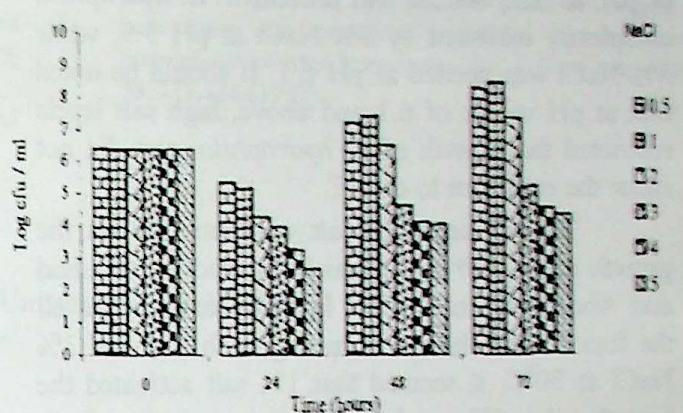
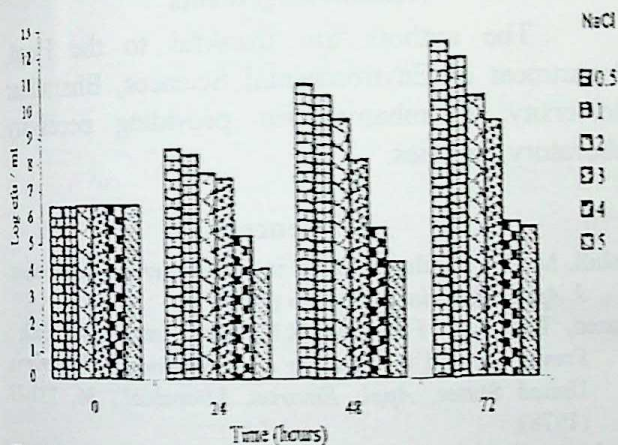
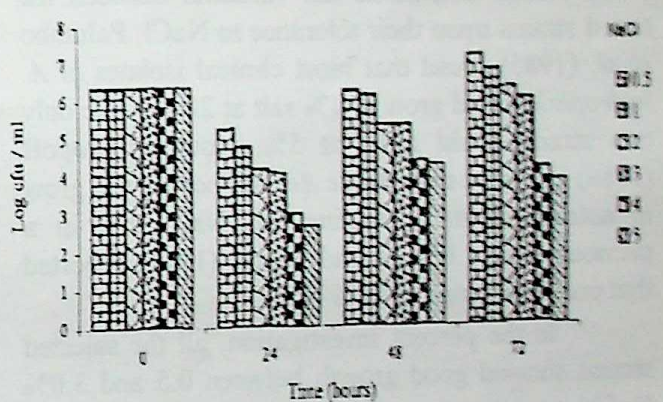
increased slightly (7.55 and 7.44 log number of cells respectively). The strains AH-86 and AH-100 did not show growth and the population observed was lower than the initial inoculum. In 5% salt concentration, no growth was observed for all the selected strains.

At 5°C, all the strains showed moderate growth in 0.5-2% salt concentrations. Number of cells ranged 8.6-7.2 log. At salt concentrations such as 3.0% 4.0% and 5.0%, none of the selected strain grew above the initial inoculum (Fig. 2e-h).

The results of the present investigation suggest the role of pH, salt concentration and temperature and their influence on the growth of *A.*

hydrophila. All the selected strains exhibited more or less similar results with respect to treatment and parameters studied.

A. hydrophila strains showed good growth at pH 7.0, 8.0 and 9.0 at both 30°C and 5°C. In acidic (5.0) and alkaline pH (10.0). *A. hydrophila* strains did not show any growth due to sudden exposure to the high acidic and high alkaline conditions, but after 24 h, they recovered and the growth gradually increased. In the present study, a delayed growth of this organism was observed at acidic pH 4.0. Palumbo *et al.* (1985) have reported the delayed growth of *A. hydrophila* at acidic pH and they grow readily in media within a wide range

Influence of environmental factors on *Aeromonas hydrophila*.Fig. 2 e: Growth of *A. hydrophila* (AH-86) in various concentrations of NaCl at 30°C.Fig. 2 f: Growth of *A. hydrophila* (AH-86) in various concentrations of NaCl at 5°C.Fig. 2 g: Growth of *A. hydrophila* (AH-100) in various concentrations of NaCl at 30°C.Fig. 2 h: Growth of *A. hydrophila* (AH-100) in various concentrations of NaCl at 5°C.

of pH at low temperature. The same authors have reported that at refrigeration (5°C), the organisms tended to be more sensitive to lowering of pH than at higher temperatures and about 9.5 log number of cells at this temperature after 14 days of incubation have been reported (Palumbo *et al.*, 1985). *A. hydrophila* strain isolated from oyster has grown in a pH range from 5-10 and yielded 11.0 log numbers at pH 7.2 and 10.0 log numbers at pH 10.0 log numbers at pH 8.5 and 9.0. At pH 6.5 and 5.5 the growth was close to 10.0 log numbers and at pH 10.0 it was nearly 9.0 log number of cells/ml (Tsai *et al.*, 1997). They have also observed to match the different initial pH in the medium. The physiological metabolism resulted in the increase of the pH to 8.4-9.0 from the initial pH 5.5.

The effect of pH on the behaviour of *A. hydrophila* at refrigeration temperature must be

considered. Hazen *et al.* (1978) concluded that pH does not seem to play a significant role in the distribution of *A. hydrophila* in natural aquatic habitats. The organism was isolated from samples having a pH range of 5.2-9.8 and its growth was unaffected at pH 5 to 9. Palumbo *et al.* (1985) reported that two out of ten strains could grow in brain heart infusion broth at pH 4.5 when incubated at 28°C for 20 days. None inhibited growth at pH 4.5 when incubated at 5°C for 24 days; for out of recorded growth at pH 5.5, eight at pH 6.5 and 9 at pH 7.2. These findings clearly indicate that *A. hydrophila* has the capability to grow at different pH values.

The authors also reported that the salt (NaCl) and pH are two traditional means of restricting the growth of food borne pathogens. When *A. hydrophila* was grown in culture media, it

was less tolerable to extremes of NaCl concentration or pH, as temperature was decreased. *A. hydrophila* completely inhibited by 2% NaCl at pH 5.9, while 3% NaCl was needed at pH 6.1. It should be noted that at pH values of 6.1 and above, high salt levels restricted the growth of *A. hydrophila*, and did not cause the organism to die off.

The influence of salt concentration on the growth of *A. hydrophila* has been reported (Rashad and Abdel-Kareem, 1995). In their study, while all the four strains showed normal growth at 1 and 3% NaCl at 30°C, it seemed that 1% salt activated the growth. At 5% and 6.5% the growth of *A. hydrophila* was arrested, however, they were viable. These results emphasize the variation between the tested strains upon their tolerance to NaCl. Palumbo *et al.* (1985) found that most clinical isolates of *A. hydrophila* could grow in 4% salt at 28°C while only two strains could grow at 5%. However, Popoff (1984) indicated that motile *Aeromonas* do not grow in nutrient broth containing 5% NaCl, but in a previous study, Popoff and Veron (1976) reported that one strain grew at 10% NaCl.

In the percent investigation, all the selected strains showed good growth between 0.5 and 3.0% NaCl concentration. At 4% salt concentration, moderate growth was observed except the strains AH-100 and AH-86. In 5% salt concentration, none of the selected organisms could grow. However, these organisms were able to tolerate this salt concentration at 30°C. At 5°C also *A. hydrophila* strains exhibited growth at 0.5% to 2.0% NaCl concentration whereas in 3.0% to 5.0% NaCl concentration, the strains did not grow but they could survive at these concentrations.

Variation in the growth of *A. hydrophila* strains due to various environmental factors could be possible because of its habitat under natural and polluted environment. In the present study, the selected strains were isolated from frozen marketed fish and prawns. The generic level identification of fish and prawns revealed that the habitat of the specimens were from marine environment. If the fish and prawns carried these organisms from the source of existence, then it is possible that the habitat of the

organisms (marine environment) favored the growth and tolerance to different NaCl concentrations.

Nowadays people prefer foods with less additives, due to this there is an increased reliance on refrigeration and it is the major factor limiting the growth of various food borne pathogens and spoilage microorganisms in food products. However, refrigeration only slows down the growth but does not prevent the growth of psychotropic food borne pathogens. Refrigerated storage should be cold enough and of duration, short enough to ensure that this bacterium does not reach levels that pose a health risk (Palumbo *et al.*, 1991; Hudson, 1992).

Acknowledgements

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Fresh water fishes as indicators of Kaveri River pollution

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Abstract : The survey of fish fauna in Kaveri River at polluted and unpolluted sites revealed a direct effect on the distribution of fishes in that 14 species were observed in unpolluted site and only 6 species in polluted site. Further, the haematological parameters like RBC, WBC and haemoglobin content increased in fishes collected from polluted site whereas the organic constituents of muscle decreased in the above fishes when compared to the fishes of unpolluted site. The reason for the above changes is discussed.

Key words : River Kaveri, Freshwater fishes, Pollution, Indicator, Haematological parameters.

Introduction

The quality of water is usually determined by its physico-chemical characteristics. It is well established fact that domestic-sewage and industrial effluents into natural water result in changes of water quality and cultural eutrophication (Shaw *et al.*, 1991). The other important sources of water pollution include mass bathing, disposal of dead bodies, rural waste-matters, agricultural run-off and solid waste disposal (Tiwana, 1992). With regard to the fisheries of the rivers, the ecology of the surface run-off water plays the most important role since ecology determines the habitability and abundance of flora and fauna in different sections (Mishra and Saksena, 1992). Fishes are very useful indices of the real state of purity of water. No river should be considered in a satisfactory condition unless fish lives and thrives in it. By observing the population of fishes, the wholesomeness of watercourse can be determined. Use of fish as indicators in water quality often serves to signify restoration of seriously polluted watercourse. Hora (1942) was first to realize that the pollution in streams is likely to affect fishes. Verma and Dalela (1975) have studies fish fauna of stressed rivers and have tried to designate fish species tolerant to pollution. To avoid undesirable toxic consequences on animals recognized sensitive monitoring systems of bio-indices should be employed in advance, to predict hazardous effects well in time. In order to conclude that a stress has occurred, a change in the rate of

physiological process must be recorded. If the animals are not normal, steady state physiology has changed, it will affect its survival and reproduction. Among the different organs of fish subjected to pollution, liver is the worst affected. Liver being the principal site of detoxification, most of the toxic substances, while passing through it may because biochemical changes (Brown, 1954). Fujiya (1961) described the biochemical changes occurring in the tissues of fish held in live boxes near a pulp and paper mill outface. Changes occurring in the biochemical characteristics of fishes provide a sensitive measure to know the health of fish fauna (Nair *et al.*, 1984).

Knowledge of the composition of blood and of the function of its components is a fundamental necessity to the understanding of the normal and pathological physiology of animal. The fish blood is an important tissue of the body, which performs most of the vital activities of the life (Saxena and Sharma, 1979). Hematological techniques including total erythrocyte and leukocyte counts have been proved valuable for fish biologists in assessing the health of fish (Bell and Margolis, 1976; Hickey, 1976). McLeay (1973), Nair *et al.* (1984) and Saravanan and Natrajan (1991) have reported the changes occurring in the haematological characteristics of fishes have been found to provide a sensitive parameter in assessing the health of fish and arriving at permissible discharge. The haemoglobin value of fish is also a useful index of

fish health (Christensen *et al.*, 1978). While assessing the physiological effect of aquatic pollutants on fish life, it is necessary to take into account many pathological changes occurring in the blood; because change in erythrocytes will impair the respiratory physiology of the fish. A careful perusal of the literature reveal that not much of work has been done relating to the distribution of fish fauna and their biochemical and blood parameters as indicator of freshwater pollution. Hence an attempt has been made to study the distribution and biochemical nature of the available fishes in the unpolluted, moderately polluted and polluted sites of the River Kaveri.

Materials and Methods

Three sampling stations of river Kaveri, for fish collection were selected on the basis of pollution (Fig. 1). The descriptions of the three distinct sampling stations are given below.

Upper Anicut (Station 1) : The station is located upstream of the first impoundment. It represents a semi-lentic system with fine and bottom. It is one of the centers of the major fishing activity of the river.

Chinthamani (Station 2) : This station is located in the heart of Tiruchirappalli City and suffers from maximum human interference. Direct discharge of sewage is an important point of concern.

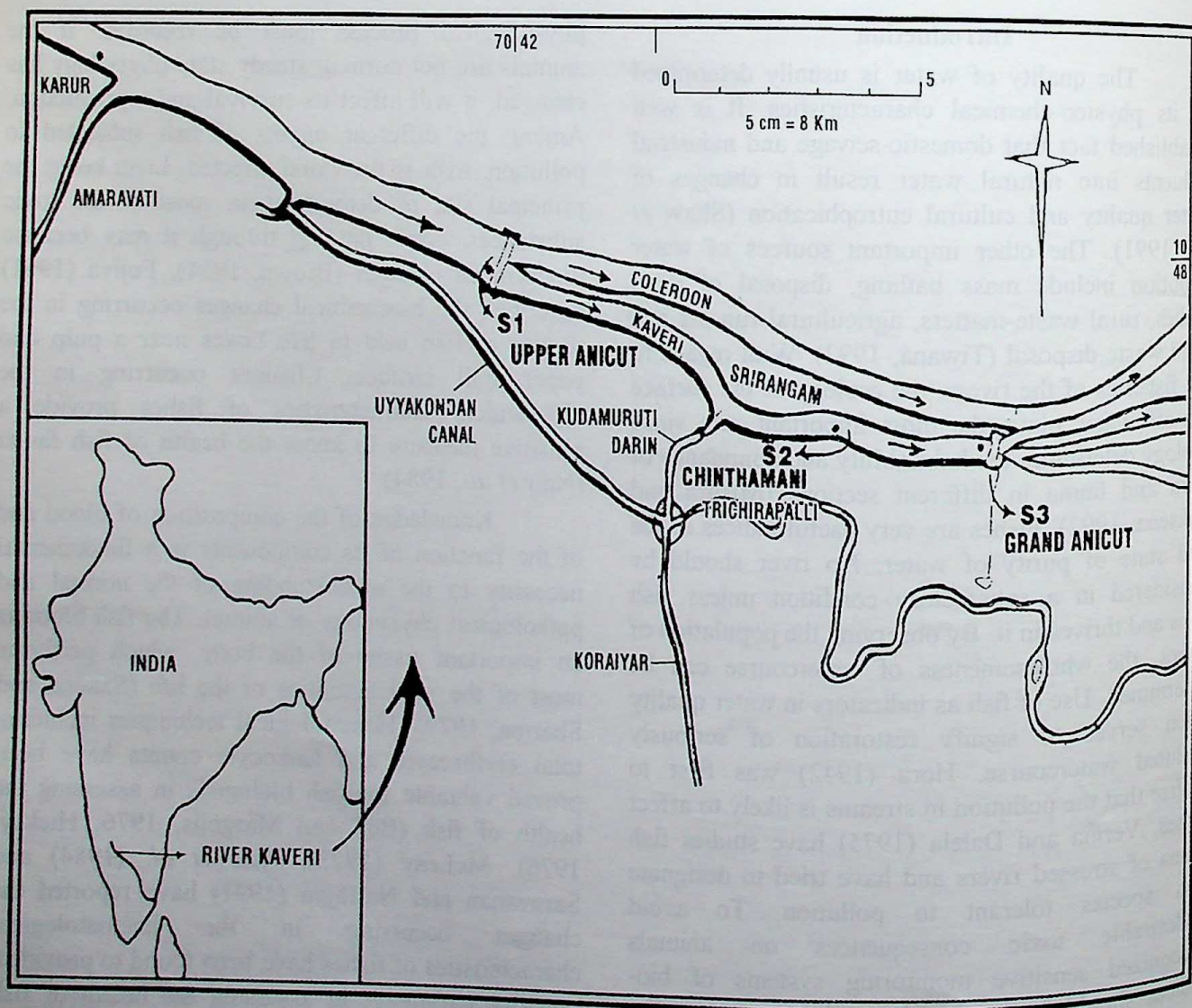


Fig. 1 : Map showing location of the sampling sites (S1, S2 and S3).

S1- Upper Anicut;

S2- Chinthamani;

S3- Grand Anicut

Fishes as indicators of river pollution.

Grand Anicut (Station 3) : It is the second impoundment across the River Kaveri. It represents a semi-lentic system with considerable pollution load received from the city. It is also one of the active fishing centres of the river.

The collection of fishes from the three different stations in the river was made with the help of cast net. At every station three nettings were done and the collected fishes were preserved in 7 % formalin solution. The pollution sensitive and pollution indicator fish species were also designated. The systematic identification of the fishes was done with the help of standard texts (Day, 1878; Jhingran, 1985). The live fishes of *Oreochromis mossambicus* and *Mystus montanus* were brought to the laboratory and sacrificed for biochemical and haematological studies. The fish were taken out and weighed after wiping the adhering water with an adsorbent paper. Blood was collected, directly from the ventral aorta by severing the caudal peduncle in to a glass vial containing a few drops of 10% Ethylene diamine tetra acetic acid (EDTA). The contents of the vial were gently mixed. The blood was used for enumerating the total numbers of RBC, WBC and to determine the haemoglobin (Hb) content. For blood cell count, Shaw's diluting fluid was used (Shaw and Burnard, 1954). By using this solution the differentiation between erythrocytes and leucocytes were readily made. Haldene's haemometer was used for determining the haemoglobin content by acid haematin method.

After the collection of blood samples, the fishes were sacrificed for tissue collection. Samples of muscle were collected from the lateral side of each and every fish. An amount of 50 mg of muscle tissue was homogenized with 80 % ethanol and centrifuged for 10 minutes at 5000 rpm. The protein, carbohydrates and lipid contents of the tissue were determined following the procedures of Lowry *et al.* (1951), Roe (1955) and Folch *et al.* (1956), respectively. The supernatant was taken for sugar estimation and the precipitate for protein estimation. Another piece of 50 mg of muscle was homogenized in chloroform : methanol mixture for lipid estimation. The statistical treatments employed

include mean difference 't' test of significance followed by Parker (1973).

Results

The effects of water quality on fish fauna in polluted and non-polluted waters in the different stations of Upper Anicut, Chinthamani and Grand Anicut in River Kaveri are presented in the Table 1 and 2.

Distribution of fish fauna : The fish community in the River Kaveri system was represented by 15 species belonging to 9 families. Relatively the fish species abundance in three different stations of River Kaveri is shown in Table 2. The highest diversity of the fish species (14 species) was observed in the station 1. The least diversity of fish species (6 species) was observed in station 2 where the domestic sewage is discharged into the river. In station 3, 11 species were recorded out of 15 species observed, under the families of *Cyprinidae*, *Bagaridae*, *Claridae*, *Heteropneustidae*, *Cichilidae*, *Channidae*, *Anabantidae*, *Anguilidae* and *Notopteridae*. In station 1, 14 species were recorded of which *Puntius* species and the major carps were dominant but other species occurred in limited numbers. In the station 2 only 7 species were recorded, and they were designated as tolerant forms due to their occurrence in this station. The dominant species in this station are *Oreochromis mossambicus*, *Anabas testudineus*, *Puntius filamentosus*, *Puntius sarana*, and *Heteropneustes fossilis*. *Heteropneustes fossilis* is commercially important species. *Puntius filamentosus*, *O. mossambicus*, *Anabas testudineus* and *Mystus vittatus* are pollution tolerant species (Palanichamy, *et al.*, 1986, Singh and Singh, 1982, Saravanan and Hari Krishan, 1997). The major carps are main pollution sensitive species. *P. filamentosus* is a pollution indicator species which is dominant in station 1. In station 2 which are less polluted by down stream of the river recorded 11 species. Out of 11 species *Channa punctatus*, *O. mossambicus*, *A. testudineus* and *M. vittatus* were dominant. *P. sarana* and *P. chola* are clear water species while other species are present in all the stations of the river and can be termed as facultative forms. Major

carps, *C. punctatus*, *H. fossilis* and *M. vittatus* have high commercial value. The Upper Anicut and Grand Anicut are the major fishing areas of River Kaveri.

The "Ghagharia net" (cast net) is mostly used for the purpose of fishing. The abundance of various species of fish depends not only on the chemical factors of

Table – 1 : The fish species and their abundance in three selected stations of River Kaveri.
[X₁=1-10%, X₂=10-25%, X₃=25-50%, *=Commercial species]

S. No.	Fish species	Upper Anicut S1	Chinthamani S2	Grand Anicut S3
	Family : Cyprinidae			
1.	<i>Cirrihinus cirrhosa</i> * (Bloch)	X ₃	---	X ₁
2.	<i>Puntius chola</i> (Hamilton)	X ₃	---	X ₁
3.	<i>Puntius filamentosus</i> (Hamilton)	X ₁	---	X ₃
4.	<i>Puntius sarana</i> (Hamilton)	X ₃	---	X ₁
5.	<i>Labeo calbasu</i> * (Hamilton)	X ₂	---	X ₂
6.	<i>Labeo rohita</i> * (Hamilton)	X ₂	---	X ₁
7.	<i>Catla catla</i> * (Hamilton)	X ₁	---	X ₁
	Family : Bagaridae			
8.	<i>Mystus vittatus</i> (Bloch)	X ₁	X ₂	X ₃
	Family : Claridae			
9.	<i>Clarius batrachus</i> * (Linnaeus)	X ₁	---	X ₁
	Family : Heteropneustidae			
10.	<i>Heteropneustes fossilis</i> * (Bloch)	---	X ₁	X ₁
	Family : Cichilidae			
11.	<i>Oreochromis mossambicus</i> (Peters)	X ₁	X ₃	X ₂
	Family : Channidae			
12.	<i>Channa punctatus</i> * (Bloch)	X ₃	---	X ₃
	Family : Anabantinidae			
13.	<i>Anabas testudineus</i> (Bloch)	X ₂	---	X ₂
	Family : Anguilidae			
14.	<i>Anguilla benalensis</i> (Gray and Hardwick)	X ₁	X ₃	---
	Family : Notopteridae			
15.	<i>Notopterus notopterus</i> (Pallas)	X ₁	---	
	Total number of species	14	6	11

Table – 2 : Fishes recorded in the selected three stations of River Kaveri for three consecutive months (December 1999 to February 2000).

River system	Order	Family	No. of species in each family
Upper Anicut	I. Cyperiniformes	1. Cyprinidae	7
		2. Bagaridae	1
		3. Claridae	1
		4. Heteropneustidae	1
Chinthamani	II. Perciformes	1. Cichilidae	1
		2. Anabantidae	1
Grand Anicut	3. Channiformes	1. Channidae	1
	IV. Osteolossiformes	1. Notopteridae	1
	V. Anguilliformes	1. Anguilidae	1
Total number of species			15

Table - 3 : Bio-chemical composition of fishes collected from Upper Anicut (S1), Chinthamani (S2) and Grand Anicut (S3) in River Kaveri. Mean \pm S.D. (n=10).

Station	<i>Oreochromis mossambicus</i>			<i>Mystus montanus</i>		
	Protein	Carbohydrate	Lipid	Protein	Carbohydrate	Lipid
1	58.54 \pm 0.60	3.07 \pm 0.29	8.02 \pm 0.66	54.91 \pm 0.37	2.85 \pm 0.12	9.84 \pm 0.31
2	56.86 \pm 0.88	1.82 \pm 0.19	7.22 \pm 0.35	50.85 \pm 0.57	1.44 \pm 0.07	8.37 \pm 0.39
3	57.77 \pm 1.15	1.77 \pm 0.12	8.25 \pm 0.17	49.62 \pm 0.85	1.48 \pm 0.18	9.09 \pm 0.49

Table - 4 : Haematological characteristics of fishes collected from Upper Anicut (S1), Chinthamani (S2) and Grand Anicut (S3) in River Kaveri. Mean \pm S.D. (n=10).

S. No.	Name of species	Stations	RBC $\times 10^6/\text{mm}^3$	WBC $\times 10^4/\text{mm}^3$	Hb g%
1	<i>Oreochromis mossambicus</i> (Peters)	1	2.10 \pm 0.08	1.11 \pm 0.05	7.90 \pm 0.56
		2	2.21 \pm 0.07	1.16 \pm 0.04	8.1 \pm 0.33
		3	2.16 \pm 0.03	1.13 \pm 0.38	8.0 \pm 0.38
2	<i>Mystus montanus</i> (Jerodon)	1	1.82 \pm 0.19	4.11 \pm 1.07	7.0 \pm 0.33
		2	1.90 \pm 0.15	4.72 \pm 0.22	7.5 \pm 0.30
		3	1.85 \pm 0.15	4.61 \pm 0.51	7.3 \pm 0.1

Table - 5 : Summary of 't' test applied for haematological and biochemical parameters of *Oreochromis mossambicus* and *Mystus montanus* selected from three different stations.

Species	Station	Haematological parameters			Biochemical parameters		
		RBC	WBC	Hb	Proteins	Carbohydrate	Lipids
<i>O. mossambicus</i>	1 vs 2	<0.01	<0.05	>0.05	<0.01	<0.01	<0.01
	1 vs 3	<0.05	>0.05	<0.05	>0.05	<0.01	<0.01
<i>M. montanus</i>	1 vs 2	>0.05	>0.05	<0.01	<0.01	<0.01	<0.01
	1 vs 3	>0.05	<0.01	>0.05	<0.01	<0.01	<0.01

the aquatic body but also on the feeding habits and chemical and physical factors.

Biochemical results : Table 3 reports the changes in the muscle protein content of *Oreochromis mossambicus* and *Mystus montanus* in the polluted and unpolluted water of River Kaveri. The protein content of *Oreochromis mossambicus* was 58.54 \pm 0.60 mg/g wet wt. in the unpolluted station (Upper Anicut), 56.87 \pm 0.88 mg/g wet wt. in the sewage polluted station (Chinthamani) and 57.77 \pm 1.15 mg/g wet wt. in station 3 which is town sewage water with moderate pollution (Grand Anicut). The muscle protein content of *Mystus montanus* was 54.91 \pm 0.37 mg/g wet wt., 50.85 \pm 0.57 mg/g wet wt. and 49.62 \pm 0.85 mg/g wet wt. in fishes collected from station 1, 2 and 3, respectively.

Average muscle protein level of *O. mossambicus* decreased from 58.54 \pm 0.60 mg/g wet wt. to 56.87 \pm 0.88 and 57.77 \pm 1.15 mg/g wet wt. in polluted and moderately polluted stations (i.e., station 2 and 3). So also *M. montanus* shows a gradual decrease in muscle protein content in the fishes collected from station 1 to 3 in that the level decreased from 54.91 \pm 0.37 mg/g wet wt. to 50.85 \pm 0.57 and 49.62 \pm 0.85 mg/g wet wt. The cholesterol level of muscle also decreased in fishes collected in three stations in both the species, the mean muscle carbohydrate level decreased from 3.07 \pm 0.29 mg/g wet wt. to 1.82 \pm 0.19 and 1.77 \pm 0.12 mg/g wet wt. in *O. mossambicus* and from 2.85 \pm 0.12 mg/g wet wt. to 1.44 \pm 0.07 and 1.48 \pm 0.18 mg/g wet wt. in *M. montanus*. The lipid content of

muscle also decreased in both species from 8.02 ± 0.66 mg/g wet wt. and 9.84 ± 0.31 mg/g wet wt. to 7.22 ± 0.33 and 8.37 ± 0.39 mg/g wet wt. in station 2 (Sewage polluted) respectively. The statistical analysis revealed significant variance occurred in all the three biochemical components of both species (Table 5).

Haematological results : The haematological parameters of fish collected from the three different stations are presented in the Table 4. In the unpolluted station (Upper Anicut), the total RBC counts were minimum for both the species of study. In *Oreochromis mossambicus*, the total RBCs are $2.10 \pm 0.08/\text{mm}^3$, $2.21 \pm 0.07/\text{mm}^3$ and $2.16 \pm 0.03/\text{mm}^3$ in Upper Anicut, Chinthamani and Grand Anicut, respectively. This result showed the maximum RBC count of the fish in the sewage polluted station (Chinthamani). In *Mystus montanus* RBC count was observed to be $1.82 \times 10^6/\text{mm}^3$, $1.90 \times 10^6/\text{mm}^3$ and $1.85 \times 10^6/\text{mm}^3$ in the fishes collected from the three stations respectively. In the station 3, the RBC count was slightly less than the sewage polluted station 2, so station 3 (Grand Anicut) was considered as a moderately polluted station. The total number of WBC of the two species was presented in the Table 4. In *O. mossambicus* WBC count was $1.11 \times 10^4/\text{mm}^3$ in the unpolluted station. There was an increase in WBC count in the sewage polluted station which is $1.16 \times 10^4/\text{mm}^3$ and in the moderately polluted station (Grand Anicut) the WBC content was $1.13 \times 10^4/\text{mm}^3$. The maximum number of WBC was noticed in the sewage-polluted station. In *Mystus montanus* the WBC content was $4.11 \times 10^4/\text{mm}^3$, $4.72 \times 10^4/\text{mm}^3$ and $4.64 \times 10^4/\text{mm}^3$ in the unpolluted, sewage polluted and moderately polluted station respectively. The WBC count was higher in the sewage-polluted station. In *O. mossambicus* the haemoglobin content was 7.90 ± 0.56 g % in the unpolluted station, which increased to 8.1 ± 0.33 g % in several polluted station but in moderately polluted station 3 (Grand Anicut), the haemoglobin content was 8.0 ± 0.38 g %, which is slightly higher than the unpolluted station. The same result was also noticed in the *Mystus montanus*. In the *M. montanus* the haemoglobin content was 7.0 ± 0.33 g %, 7.5 ± 0.30 g % and 7.3 ± 0.51 g % in fishes

collected from the station 1, 2 and 3, respectively. There are significant variance occur in blood parameters of the both species (Table 5).

Discussion

Population explosion has the main role on the discharge of municipal sewage in the river. Discharge of industrial effluent, agricultural and domestic sewage from various sources has posed a threat for human health, survival of fish and other aquatic organisms. Katz and Gauvin (1952) described that while designing species of fish as indicator of pollution, no single species of fish should be considered, instead the number of species available at particular place and their relative abundance should be considered. Eighty species of fishes belonging to 23 families have been reported by Chacko *et al.* (1954) in River Kaveri. In a survey of Kali river conducted by George *et al.* (1966), it was observed that carps, namely *Labeo rohita*, *L. dero*, *Catla Catla*, *Cirrihinus mrigala* and *Puntius sarana* and cat fishes like *Wallago attu*, *Rita rita*, *Bagarius bagarius*, *Mystus seenghala* and *M. oar* were most common. *Ophiocephalus*, *Heteropneustes*, *Clarias* and *Notopterus* were least affected by pollution in the river. Fishes of Narmada River are represented by about 23 species of fishes, 10 belonging to carp, 8 to cat fish, 2 to murrel group, 2 to spiny-eel and 1 to feather-backs (Karamachandani *et al.*, 1967). In Jamuna River, Prakash *et al.*, (1978) have collected 95 species of fishes with abundance of cyprinoid fishes. They have noted that *Notopterus*, *Puntius*, *Catla* and *Labeo* occurred throughout the year and *Clarias batrachus* and *Lepidocephalicthys* genera were rarely noted.

The present study on fish abundance and diversity of River Kaveri has shown that the sewage polluted river area has poor fish fauna consisting (6 species) in station 3. In this station major carps were completely absent. *Puntius filamentosus*, *Mystus vittatus*, *O. mossambicus* and *Anabas testudineus* were dominant. *Heteropneustes fossilis* also recorded in this station rarely in one collection. *O. mossambicus* and *A. testudineus* are resistant species and commonly found in polluted waters. *P. chola*

Fishes as indicators of river pollution.

and *P. sarana* are highly sensitive and not recorded in this station.

The tolerance of *O. mossambicus* in Coovum River is also observed by Joseph *et al.* 1992. In station 1, 14 fish species were recorded. This station is non-polluted and represents a maximum number of species. The major carps and other species like *H. fossilis*, *C. batrachus*, *M. vittatus* and *C. punctatus* were observed in this station. In station 3, which is moderately polluted, represents 11 species. All the pollutants discharged into the middle of the river are completely diluted when they reach this station. In this station *P. filamentosus*, *C. punctatus*, *A. testudineus* and *O. mossambicus* were dominant. All the other species are least occurred. The rich abundance (14 species) observed in non-polluted (station 1), was also observed by Banerjee and Motwani (1960). In general, species composition and species richness are the biological parameters most attracted by anthropogenic pollution sources and therefore biological indices should be sensitive to alteration in these two parameters. River Kaveri has rich source of a fish species, except the sewage polluted station. Generally the River Kaveri is least affected by pollutants and it has a rich fish composition and abundance. In the present investigation biochemical and haematological parameters were studied in *O. mossambicus* and *M. montanus* collected from the polluted and unpolluted stations of River Kaveri. It was noted that there is a decrease in protein, carbohydrates and lipid contents in the fishes collected from polluted station. The decrease is more in polluted and less in moderately polluted sites. This is due to the pollution stress posed to the fishes. Carbohydrate represents the principle and immediate energy source for fish exposed to stress condition and protein also shared a major role (Umminger, 1970). In the present study also the carbohydrate content showed a decrease in the two fishes collected from sewage and other polluted stations. The reduction in muscle carbohydrate can be attributed to its utilization for the increased energy demand posed by the pollution stress. It is possible to suggest that the depletion of protein in muscle could be due to the mobilization of protein from muscle to blood, to

compensate, to certain acidosis caused by the lactate accumulation. Palanichamy *et al.* (1986), Saravanan and Harikrishan (1997, 1998) have reported that depletion in protein level was due to the diversification of energy to meet the impending energy demands when the animal was under stress. In fact, Saravanan *et al.* (2000) have reported the nutritive loss of the fish, *O. mossambicus* on exposure to endosulfan for a period of 6 weeks.

Lipid is an important fuel reserve of the fish during stress situation it is mobilized to meet the energy needs. As a result of change in the carbohydrate metabolism during stress condition there will be a change in the lipid content of the animal. Decline in lipid content was noticed in *O. mossambicus* and *M. montanus* collected from industrial effluent discharging stations and sewage disposing station. Konda *et al.* (1973) reported a decrease in lipid and protein in the liver of *H. fossilis* exposed to vegetable oil factory effluent. Gunstone (1960) suggested that the decline in the lipid content may be due to either oxidation or hydrolysis of lipid. According to Fry (1969) the fishes resist a changed situation for a specific period but will eventually susceptible as a result of their inability to continuously adapt. Generally the haematological parameters in a fish reflect the ecological condition of its habitat.

In the present investigation among two species, there is an increase in red blood corpuscles, white blood corpuscles and haemoglobin in the down stream of the sewage polluted station than the control which is said to be unpolluted station. Haniffa *et al.* (1986) reported that RBC of *H. fossilis* and *O. mossambicus* increased after exposure to distillery and paper mill effluents. In the present study, there was increase in the RBC in *M. montanus* collected from Chinthamani and Grand Anicut. The same was reported by Isaiarasu and Haniffa (1985) in *M. montanus* when exposed to distillery and paper mill effluent, so also Saravanan and Natrajan (1991) have reported significant changes in haemoglobin parameters on *A. testudineus* on exposure to cadmium. In fact, McLeay (1973) stated that in *O. kistutch*, erythropoiesis was stimulated when exposed to pulp mill effluent. This was due to the

elevated demands for oxygen and CO₂ transport in the polluted medium. He also reported that erythropoiesis could also be stimulated by an increased fragility or rate of destruction of circulating erythrocytes. Both haematological and bio-chemical parameters referred significantly in fish obtained from polluted station (Table 5). In the present investigation too, this was confirmed in the species collected from the station where the sewage is discharged.

Thus, the present study revealed significant reduction in the number of fish species in polluted stations when compared to unpolluted station. The tissue metabolite also decreased in fishes collected from polluted stations revealing stressed condition. As a confirmation even the haematological parameters showed significant alterations in fishes exposed to polluted station. Thus the freshwater fishes serve as bio-indicators of the pollution in the River Kaveri.

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Insecticidal activity of the plant *Phyllanthus amarus* against *Tribolium castaneum*

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Abstract : The plant *Phyllanthus amarus* is used as folk medicine since the year 1800 and has been established for its important medicinal properties particularly for liver ailments. The present communication explores the insecticidal activity of ethanolic extract of aerial and root parts of this plant against stored grain pest *Tribolium castaneum*. LC₅₀ values of ethanolic aerial part were 895.77, 473.91, 279.89 and 260.85 $\mu\text{g}/\text{cm}^2$, while 512.62, 376.96, 248.88 and 209.79 $\mu\text{g}/\text{cm}^2$ for ethanolic root part at the exposure of 3, 5, 7, 9 and 11 days respectively. Ethanolic root extract possessed significant insecticidal activity against *T. castaneum*.

Key words : Biopesticide, *Phyllanthus amarus*, Insecticidal activity, *Tribolium castaneum*.

Introduction

Food grains are potentially prone to different insect pests and among them *Tribolium castaneum* Herbst is the most common. The insect pest causes direct and indirect losses both in quality and quantity during storage. About 70,000 species of insects and mites attack different parts of agricultural plants during growth and in storage. Use of insecticides is one of the most important components in pest management. However, environmental and human hazards of conventionally used synthetic pesticides are now well recognized. As a result, the scientists world over are turning to ecologically sound pest control technologies specially based on plant-based pesticides. The plant kingdom offers a rich storehouse of chemicals with diverse biological effects on insects. In recent years, several plants have been identified, which can be used as renewable source of insecticides (Duke, 1990; Sharma *et al.*, 1992; Dev and Koul, 1997) and are reported to be comparatively safe to mammals and higher animals (Kashyap *et al.*, 1992; Rao and Panwar, 1996).

In continuation of our work on insecticidal activity of the plant *Phyllanthus amarus* against mosquito larvae (Khanna *et al.*, 2000), the present investigation explores the possibility of a viable herbal alternative for prevention of stored grain damage against the most serious stored grain pest

Tribolium castaneum. The plant *Phyllanthus amarus* has been very well characterized for its medicinal properties. Its herbal extract is used for liver ailments (Sane *et al.*, 1997), hepatitis B (Mehrotra *et al.*, 1990), diabetes (Moshi *et al.*, 1997) and urinary disorders (Srividya and Periwal, 1995).

Materials and Methods

Plants were collected from Dayalbagh Educational Institute, Agra Campus during August - September 1999. Plant samples were authenticated and deposited in the herbarium of National Botanical Research Institute, Lucknow (Accession No NBRI 89381). Plants were partitioned into aerial and root parts and dried in a shady place in the laboratory. The shade dried powdered plant parts were subjected for soxhlet extraction successively with petroleum ether and 95% ethanol. The residual portion obtained after removing the respective solvents (rotary evaporator) was dried by purging nitrogen. Colored waxy mass obtained from different solvents was weighed accurately. The target species (*T. castaneum*) were reared and maintained on sterilized wheat flour fortified with 5% brewer's yeast at $35 \pm 2^\circ\text{C}$ and $70 \pm 5\%$ relative humidity. Different dose concentrations were prepared from stock solution using distilled acetone. Insecticidal activity was observed in the petridishes (diameter 4 cm) having greasy inner wall. At the bottom of the inner surface

of petridishes, doses were spread over and were warmed on the water bath to remove solvent completely. Solvent free petridishes having dose concentration and wheat flour, twenty adult *T. castaneum* were transferred and covered. Three replicates were set up for each dose against control. Mortalities encountered at each dose concentration at exposure period of 3, 5, 7, 9 and 11 days were recorded. LC_{50} was determined based on probit analysis method (Finney, 1971).

Results and Discussion

The effectiveness of the extracts was determined based on percent mortality of the target species as a function of time. Various parameters were used for the determination of LC_{50} using probit analysis method. Values were further checked for their 95% confidence limit, regression equation and heterogeneity.

Petroleum ether extract of aerial and root part did not show any noticeable insecticidal activity. Ethanolic extract of aerial and root parts showed insecticidal activity against target species. In case of

ethanolic aerial extract, hundred percent mortality was observed at test dose of 795.54 and 1193.31 $\mu\text{g}/\text{cm}^2$ at 9th day of exposure (Fig. 1) while for ethanolic root extract, hundred percent mortality was recorded at test concentration of 477.32 and 397.77 $\mu\text{g}/\text{cm}^2$ at the exposure of 9th and 11th day, respectively (Fig. 2).

LC_{50} versus time variations of ethanolic aerial extract recorded for target insect species show initially a sharp decrease in LC_{50} as function of time (3-7days) followed by a gradual decreasing trend upto maximum time exposure (11th day). LC_{50} values were 895.77, 473.91, 279.89 and 260.85 $\mu\text{g}/\text{cm}^2$ at exposure of 3, 5, 7, 9 and 11 days respectively. LC_{50} values versus time variations of ethanolic root extract observed for *T. castaneum* show a continuous decreasing trend. LC_{50} values were 512.62, 376.96, 248.88 and 209.79 $\mu\text{g}/\text{cm}^2$ at the exposure of 3, 5, 7, 9 and 11 days, respectively (Fig. 3). Heterogeneity for aerial ethanolic extract were 0.438, 0.249, 0.316, 0.008 and 0.002 while 0.438, 0.249, 0.316, 0.008 and 0.000 for root ethanolic extract at the exposure of 3, 5, 7, 9 and 11

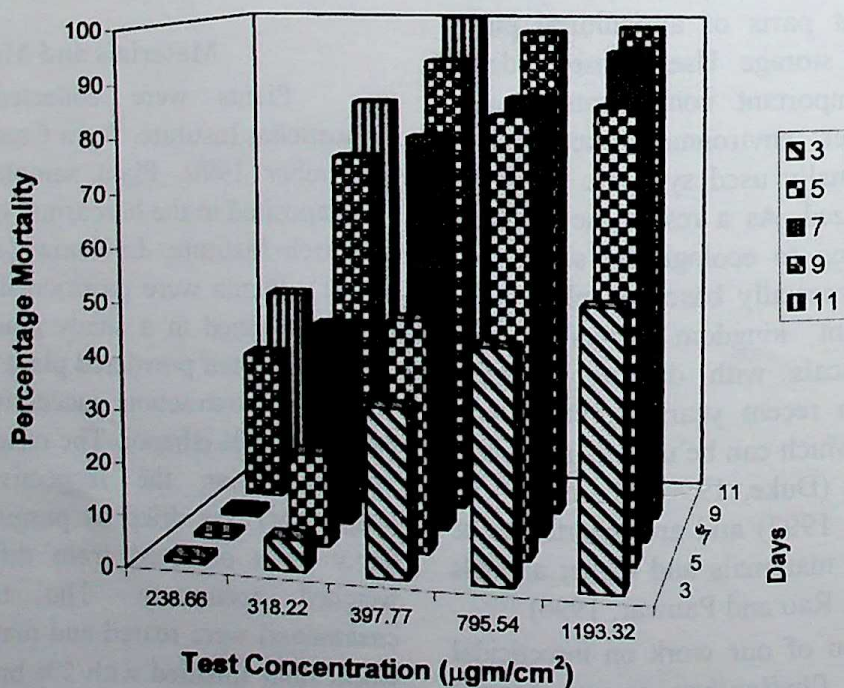


Fig. 1 : Per cent mortality versus dose and time exposure of ethanolic aerial extract against *Tribolium castaneum*.

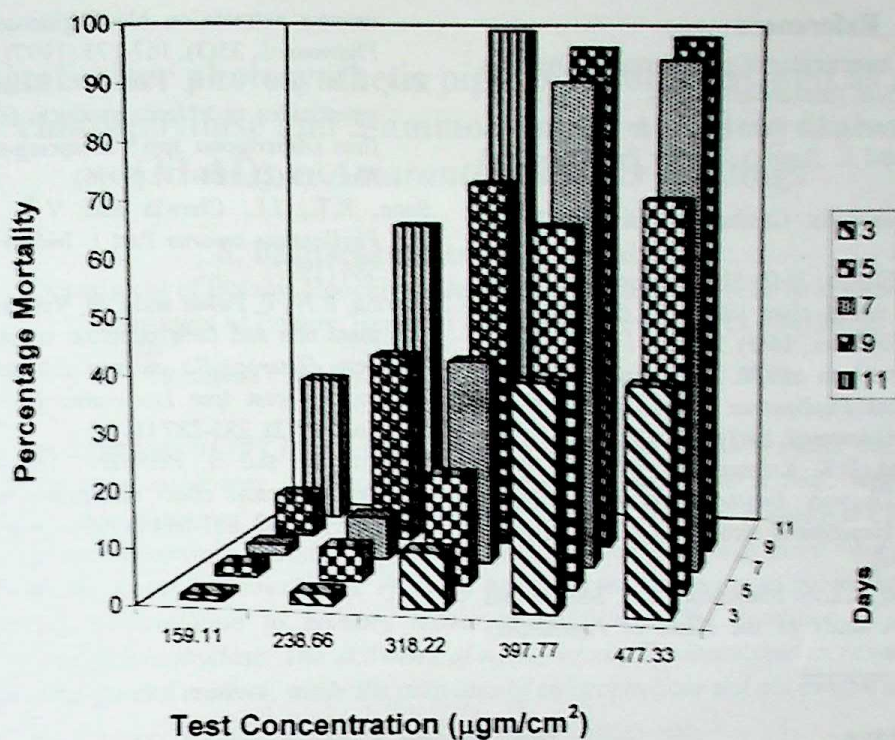
Insecticidal activity of the plant *Phyllanthus amarus*.

Fig. 2 : Per cent mortality versus dose and time exposure of ethanolic root extract against *Tribolium castaneum*.

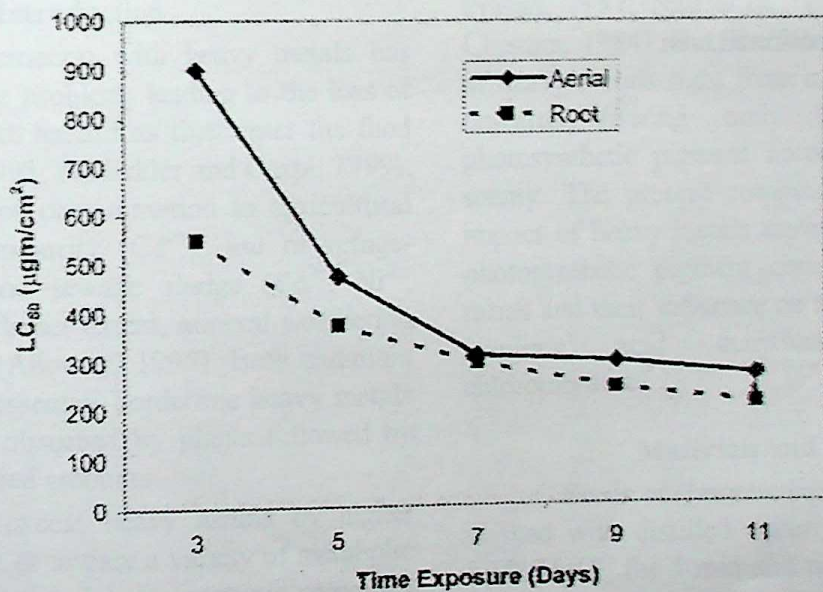


Fig. 3 : LC₅₀ versus time exposure of ethanolic aerial and root extract against *Tribolium castaneum*.

days, respectively. Between the two ethanolic extracts (aerial and root), root extract showed more potential insecticidal activity against *T. castaneum*.

Our Bioassay oriented evaluation, underscores that ethanolic root extract is quite active against the target species and requires the chemical characterization of the bioactive principle. The work is in progress on these lines.

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Heavy metals alter photosynthetic pigment profiles as well as activities of chlorophyllase and 5-aminolevulinic acid dehydratase (ALAD) in *Amaranthus lividus* seedlings

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Abstract : Varied concentrations of $PbCl_2$ and $CdCl_2$ in the germinating media reduced the total chlorophyll and carotenoid contents in primary leaves of *Amaranthus lividus* seedlings (168 h old). When chlorophyll a and chlorophyll b contents were measured separately, greater loss of chl b than chl a under the identical conditions of heavy metal treatment was observed. In addition, the loss of total chlorophyll was more than carotenoids under the same magnitude of heavy metal treatment. The effect of heavy metal treatment at germination stage was further studied on chlorophyll accumulation in primary leaves in relation to the activities of 5-aminolevulinic acid dehydratase (ALAD) and chlorophyllase. The activities of ALAD gradually diminished in response to both the heavy metals in a concentration-guided manner, while the activities of chlorophyllase did not exhibit any significant change.

Key words : *Amaranthus*, ALAD, Chlorophyllase, Cadmium, Lead.

Introduction

Soil contamination with heavy metals has become a worldwide problem, leading to the loss of crop yield and health hazard as they enter the food chain (Salt *et al.*, 1995; Schlickler and Caspi, 1999). The main sources of contamination in agricultural soil are fertiliser impurity (Cd^{2+}), use of refuge-derived compost and sewage sludge (Cd^{2+} , Ni^{2+} , Pb^{2+} , etc.) and to a lesser extent, mineral weathering (Hildebrand, 1989; Alloway, 1995). Both cadmium and lead are non-essential borderline heavy metals and are effectively absorbed by plants followed by accumulation in varied amounts.

Uptake of excess heavy metals by higher plants can modulate or initiate a variety of metabolic reactions, leading to the global phytotoxic responses like stunted growth, chlorosis, etc. (Vangronsveld and Clijsters, 1984). Heavy metals can directly or indirectly interfere with the cellular metabolism as well as cellular components and structures, inhibiting various physiological processes (Shah and Dubey, 1995; Vangronsveld and Clijsters, 1984; Satakopan and Rajendran, 1989). Heavy metals influence biosynthesis of photosynthetic pigments and structural components of chloroplasts (Prasad and

Prasad, 1987; Nag *et al.*, 1981; Vangronsveld and Clijsters, 1984). But, the studies regarding the effect of heavy metals right from early imbibitional period onwards (during early germination) on the photosynthetic pigment accumulation are relatively scanty. The present communication deals with the impact of heavy metals during early germination on photosynthetic pigment compositions, their altered ratios and their influence on the activities of 5-amino levulinic acid dehydratase (ALAD) and chlorophyllase.

Materials and Methods

Seeds of *Amaranthus lividus* Linn were first washed with distilled water, surface sterilized with 0.1% $HgCl_2$ for 5 min and washed twice with sterile distilled water for 15 min and then allowed to imbibe water for 4 h. Water imbibed seeds were then germinated in petridishes on filter papers soaked with solutions of $PbCl_2$ and $CdCl_2$ of different concentrations (0, 1, 10, 100, 1000 μM) in dark ($24 \pm 2^\circ C$, RH 78-80%) for 7 days.

Photosynthetic pigments were extracted and estimated following the method of Litchenthaler and Welburn (1983). For the extraction of photosynthetic

pigments, 50 mg primary leaf tissues (168 h old treated and untreated seedlings) were homogenized with 5 ml of 96% ethanol and then centrifuged at 5000 rpm for 10 min. The absorbance was measured at 665, 649 and 470 nm by UV-VIS spectrophotometer (Beckman DU 64). The pigment contents were calculated using the following formulae :

$$\text{Chl a} = (13.95 \times A_{665}) - (6.88 \times A_{649}) \mu\text{g ml}^{-1}$$

$$\text{Chl b} = (24.96 \times A_{649}) - (7.32 \times A_{665}) \mu\text{g ml}^{-1}$$

$$\text{Carotenoids} = \frac{(1000 \times A_{470}) - [(2.05 \times \text{chl a}) - (11.48 \times \text{chl b})]}{245} \mu\text{g ml}^{-1}$$

Chl a, chl b, total chlorophyll (chl a + chl b) and carotenoids were then expressed in terms of mg g⁻¹ dry matter (d.m.).

ALAD was extracted and estimated according to Schneider (1970). Activity was expressed in terms of nmol prophobilinogen (PBG) min⁻¹ g⁻¹ d.m. For extraction and estimation of chlorophyllase, process of Nag *et al.* (1981) was followed. The enzyme activity was expressed as % chl degraded g⁻¹ d.m. min⁻¹.

Results and Discussion

With the increasing concentrations of both CdCl₂ and PbCl₂, chlorophyll a, b and carotenoid amounts decreased in primary leaves of 168 h old seedlings (Table 1). Decreasing trends of all the pigments were evident with gradual increase of heavy metal concentrations from 1 μM to 1000 μM, and the maximum inhibition was observed for seedlings raised at 1000 μM CdCl₂ treatment. So, the decreasing trends of the photosynthetic pigments were definitely guided by the concentrations of heavy metals. Cadmium seemed to be more detrimental than lead as far as pigment accumulation was concerned, under the same magnitude of stress. The results clearly established that heavy metals could influence the biosynthesis of photosynthetic pigments.

When the chlorophyll a/b ratio was measured, it showed slight increase over control suggesting more loss of chlorophyll b than

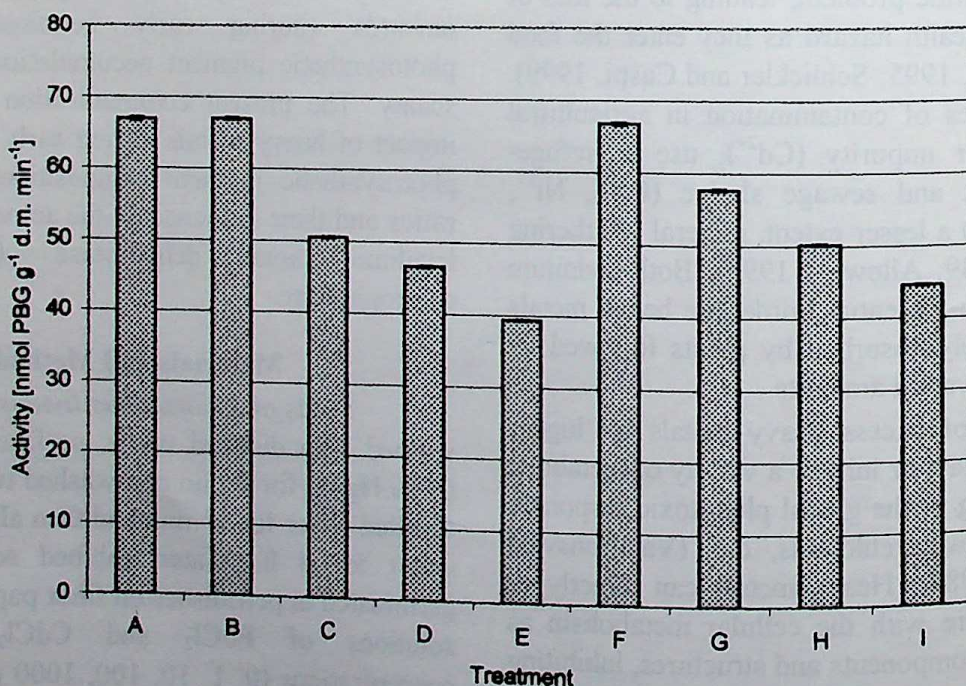


Fig. 1 : Effect of CdCl₂ and PbCl₂ (germination stage treatment) on 5-amino levulinic acid dehydratase (ALAD) activity in primary leaves of *Amaranthus lividus* seedlings (168 h old). Values are mean of four replicates. Error bars represent \pm SE.

A : untreated B : 1 μM CdCl₂ C : 10 μM CdCl₂ D : 100 μM CdCl₂ E : 1000 μM CdCl₂
 F : 1 μM PbCl₂ G : 10 μM PbCl₂ H : 100 μM PbCl₂ I : 1000 μM PbCl₂

Heavy metals on photosynthetic pigments.

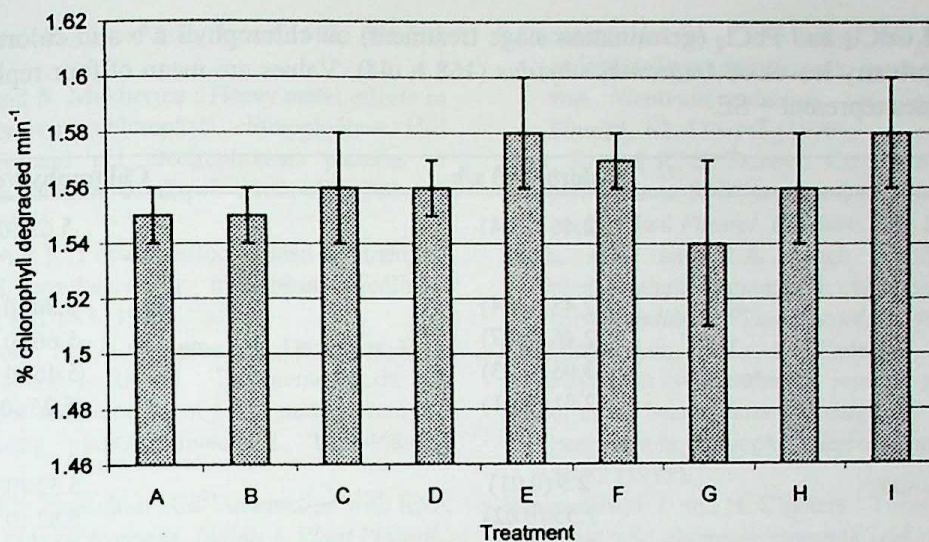


Fig. 2 : Effect of CdCl_2 and PbCl_2 (germination stage treatment) on chlorophyllase activity in primary leaves of *Amaranthus lividus* seedlings (168 h old). Values are mean of four replicates. Error bars represent \pm SE.

A : untreated B : 1 μM CdCl_2 C : 10 μM CdCl_2 D : 100 μM CdCl_2 E : 1000 μM CdCl_2
 F : 1 μM PbCl_2 G : 10 μM PbCl_2 H : 100 μM PbCl_2 I : 1000 μM PbCl_2

Table - 1 : Effect of CdCl_2 and PbCl_2 (germination stage treatment) on photosynthetic pigment compositions of primary leaves of *Amaranthus lividus* (168 h old). Values are mean of four replicates. Values in parentheses do represent \pm SE.

Treatment	Pigments (mg g^{-1} dry mass)			
	Chlorophyll a	Chlorophyll b	Carotenoids	Total chlorophyll
Untreated	4.85 (0.1)	1.97 (0.01)	1.21 (0.01)	6.82 (0.17)
CdCl_2				
1 μM	4.93	1.98 (0.010)	1.19 (0.02)	6.91 (0.14)
10 μM	4.47 (0.10)	1.81 (0.02)	1.11 (0.01)	6.28 (0.18)
100 μM	3.51 (0.09)	1.08 (0.01)	0.84 (0.01)	4.59 (0.11)
1000 μM	2.74 (0.04)	0.94 (0.01)	0.70 (0.01)	3.68 (0.09)
PbCl_2				
1 μM	4.86 (0.06)	1.94 (0.01)	1.23 (0.02)	6.80 (0.04)
10 μM	4.73 (0.08)	1.85 (0.01)	1.13 (0.01)	6.58 (0.11)
100 μM	3.70 (0.10)	1.17 (0.02)	0.98 (0.02)	4.87 (0.12)
1000 μM	3.02 (0.04)	1.00 (0.01)	0.82 (0.03)	4.02 (0.13)

chlorophyll a under the identical conditions of heavy metal treatment (Table 2). On the other hand, when the ratio of chlorophyll/carotenoid was examined in seedlings raised from varied treatments with CdCl_2 and PbCl_2 , a little decline in the concentration range of 100 μM and 1000 μM was observed, hinting at more loss of total chlorophyll than carotenoids. Inhibition of chlorophyll biosynthesis by heavy metals was described for higher plants by various workers

(Vangronsveld and Clijsters, 1984; Prasad and Prasad, 1987; Nag *et al.*, 1981). Impaired chlorophyll biosynthesis under the influence of heavy metals might be due to interference of structural components of chloroplasts. Imbibitional treatment with cadmium and lead presumably block the syntheses of enzymic proteins responsible for photosynthetic pigment biogenesis (Nag *et al.*, 1981; Bhattacharjee and Mukherjee, 1994).

Table - 2 : Effect of CdCl_2 and PbCl_2 (germination stage treatment) on chlorophyll a/b and chlorophyll/carotenoid ratio of primary leaves of *Amaranthus lividus* (168 h old). Values are mean of four replicates. Values in parentheses represent \pm SE.

Treatment	Chlorophyll a/b	Chlorophyll/carotenoid
Untreated	2.46 (0.04)	5.63 (0.03)
CdCl_2		
1 μM	2.48 (0.04)	5.80 (0.06)
10 μM	2.46 (0.07)	5.66 (0.07)
100 μM	3.05 (0.03)	5.46 (0.03)
1000 μM	2.91 (0.01)	5.25 (0.14)
PbCl_2		
1 μM	2.5 (0.01)	5.52 (0.11)
10 μM	2.55 (0.02)	5.82 (0.14)
100 μM	3.16 (0.07)	4.96 (0.14)
1000 μM	3.02 (0.01)	4.90 (0.10)

Experimental data for the reduction of chlorophyll content with the concomitant inhibition of photosynthesis by treatment with Hg, Cu and Zn are available with unicellular chlorophyll biogenesis; the increased activities of lipoxygenase might also contribute to the decreased level of chlorophyll with heavy metal treatment (Somashekaraiah *et al.*, 1992). It is interesting to note that among the major photosynthetic pigments, carotenoids were least affected by heavy metals. Cadmium and lead, as other chemical stresses might affect chlorophyll metabolism adversely by weakening the binding force between pigment protein and lipid in chloroplast structure (Singh and Singh, 1999).

The influence of Cd^{2+} and Pb^{2+} on chlorophyll metabolism in primary leaves of *Amaranthus* seedlings was further investigated in the context of the activities of ALAD and chlorophyllase (Figs. 1 and 2). The activities of ALAD decreased gradually with increasing concentrations of CdCl_2 and PbCl_2 . Maximum decrease was for the seedlings raised from 10 μM CdCl_2 treatment. On the contrary, the activity of chlorophyllase could not exhibit any significant changes for the seedlings treated with CdCl_2 and PbCl_2 (1 μM and 10 μM). The activities of chlorophyllase were slightly elevated for the seedlings when raised from 100 μM and 1000 μM cadmium and lead chloride treatments. The inhibitory effect of both the heavy metals might

be related to decrease in chlorophyll biosynthesis rather than degradation. ALAD is a metal sensitive and thiol dependent enzyme of chlorophyll biosynthesis (Jain *et al.*, 1996; Nag *et al.*, 1981) and the possible reduction of activity in response to heavy metals could be due to their impact on thiol group of the functional enzyme ALAD (Jain *et al.*, 1996; Prasad and Prasad, 1987).

So, in essence, heavy metals Cd^{2+} and Pb^{2+} treatments (starting from early imbibitional period onwards) potentiates a significant retarding effect on photosynthetic pigment metabolism and accumulation, which may be one of the significant causes of impaired seedling establishment and subsequent plant growth.

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Effect of mercuric chloride on circulating hormones in adult albino rats

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Abstract : The effect of mercuric chloride at two different doses, 0.5 mg/kg body weight (low dose), 1 mg/kg body weight (high dose), for 30 days, was seen on the circulating hormones in the mature male albino rats. Testosterone level was markedly decreased in the low dose ($P < 0.01$) and high dose ($P < 0.001$) treated animals. The level of luteinizing hormone (LH) was also reduced in the low dose ($P < 0.01$) as well as in the high dose ($P < 0.001$) treated animals. However, follicle stimulating hormone (FSH) and prolactin (PRL) levels were found to be decreased only in the high dose ($P < 0.05$) treated animals and no change was observed in the low dose treated animals. The changes in the hormone levels caused by the mercuric chloride treatment suggest the dysfunction of pituitary-testicular axis.

Key words : Mercuric chloride, Rat, Testosterone, LH, FSH, Prolactin.

Introduction

It is now evident that toxic substances like heavy metals released into the environment affect the reproductive processes and fertility of the animals. These toxic metals may act directly or indirectly on reproductive system. Toxic metals can attack the male reproductive system at one of several sites or at multiple sites. Primary targets include the neuroendocrine system, testis, accessory sex organs and sexual function (Schrader, 1997). Mercury is a direct acting toxicant. It is evident from several studies that mercury passes through the blood-epididymal barrier as well as blood-testis barrier to affect spermatogenesis and steroidogenesis (Chowdhury *et al.*, 1986; Rao, 1989).

It is evident that toxic pollutants like metals are known to accumulate in the testicular interstitial regions thereby impairing the Leydig cell activity. Mercuric chloride administration leads to the intercellular accumulation of mercury in the interstitial Leydig cells as well as in Sertoli cells of the seminiferous tubules in the testis of rat (Ernst *et al.*, 1991). Mercury elicited direct toxic action on steroid producing cells in the adrenal gland and the testis *in vitro* (Ng and Liu, 1990). LH and testosterone levels provide an index of Leydig cell function. Testicular damage associated with Leydig cell dysfunction is reflected in the low level of serum testosterone (Vouk and Sheehan, 1983).

The endocrine system in concert with the nervous system coordinates the function of various components of the reproductive axis. Hypothalamus directly regulates gonadotrophin secretion by the anterior pituitary gland. The gonadotrophins, in turn, act upon Sertoli cells, germ cells and Leydig cells to regulate spermatogenesis and hormone production by the testis (Schrader, 1997). The endocrine function of testis is reflected in serum levels of gonadotrophins and androgen. Both LH and FSH are necessary for normal spermatogenesis. LH initiates steroid secretion and release of mature gametes (Rommerts *et al.*, 1983). Testosterone has its effect not only on the testis but also on the accessory sex organs and sexual function.

Therefore the present study was planned to investigate the effect of mercuric chloride on serum testosterone and gonadotrophins as reflective of its impact on steroidogenesis and thereby on male reproduction.

Materials and Methods

90 days old male albino rats (Wistar strain) were used in the present investigation. They were maintained in standard laboratory conditions. They were housed in a room with controlled temperature ($25^{\circ} \pm 1^{\circ}\text{C}$), humidity ($50 \pm 5\%$) and lighting (12 hr light and 12 hr dark). The rats were fed with standard commercial dry food pellets and water *ad libitum*.

After 10 days adaptation period, the rats were divided into three groups and each group consisted of 15 animals.

Group I : Control rats-given 0.5 ml of distilled water as vehicle orally, daily for 30 days

Group II : Experimental rats-low dose-given mercuric chloride, 1 mg/kg body weight (in 0.5 ml distilled water) orally, daily for 30 days

Group III : Experimental rats-high dose-given mercuric chloride 2.0 mg/kg body weight (in 0.5 ml distilled water) orally, daily for 30 days

The dose was selected based on the previous study from our laboratory (Ramalingam *et al* 2002 a, b)

At the end of experimental period 10 rats were randomly selected from each group and weighed. They were killed under pentobarbital anesthesia (5 mg/kg) and blood was collected in heparinised tubes by cardiac puncture. The plasma obtained by centrifugation at 3000 rpm was stored at -20° C and used for hormone assays.

Plasma FSH, LH and PRL were measured in duplicate using RIA kits supplied by National Hormone and Pituitary Program (NHPP). NHPP rat FSH (rFSH-RP-2; rFSH-1-9 and anti rFSH-S-11), rat LH (rLH-RP-3; rLH-1-9 and anti rLH-S-11) and rat prolactin (rPRL-RP-3; rPRL-1-6 and anti rPRL-S-9) were used for these assays.

The detection limit was 1.98 ng/ml for FSH, 0.31 ng/ml for LH and 1.94 ng/ml for PRL. The intra and inter assay coefficients of variations were 7 and 8 % for FSH, 7 and 9 % for LH and 8 and 9% for PRL, respectively.

Plasma testosterone was measured in duplicate by RIA, using the reagents from a commercial kit (DPC, USA). The detection limit was

0.16 ng/ml and intra and inter assay variations were 9 and 10 %, respectively.

The data were analyzed by Student's 't' test.

Results and Discussion

The serum hormone level after the administration of mercuric chloride is depicted in Table 1. Testosterone level was markedly decreased in low dose ($P < 0.01$) as well as in the high dose ($P < 0.001$) treated animals. LH level was also decreased in low dose ($P < 0.01$) and in high dose ($P < 0.001$) group. However, FSH and PRL levels were decreased only in the high dose treated animals ($P < 0.05$) and no significant change was observed in the low dose treated animals.

The data on serum hormones in the present study reveals some interesting findings. In general it suggests the dysfunction of the hypothalamo-hypophyseal-testicular axis. Under normal conditions when the serum testosterone level was consistently low, serum LH level may be expected to be high through negative feed back mechanism. However, this was not the case in the present study. Along with the decrease in serum testosterone, serum LH level was also reduced markedly due to mercuric chloride treatment.

LH mainly regulates steroidogenesis in Leydig cell. LH binds to specific receptors on the Leydig cell membrane leading to an increase in intracellular cAMP (Dufau and Catt, 1978; Hall, 1988). The acute effect of LH on cAMP is to increase the transport of cholesterol to the inner mitochondrial membrane by a sterol carrier protein-2 with the cholesterol side chain cleavage enzyme, which catalyses the conversion of cholesterol to pregnenolone (Miller, 1988; Payne, 1990).

Table-1 : Effect of mercuric chloride on circulating hormones in adult albino rats.

Hormones	Control	Low dose	High dose
Testosterone (ng/ml)	2.52 ± 0.156	1.64 ± 0.135 ^b	0.94 ± 0.149 ^c
LH (ng/ml)	0.764 ± 0.039	0.406 ± 0.036 ^b	0.266 ± 0.03 ^c
FSH (ng/ml)	7.42 ± 0.271	7.34 ± 0.526	6.28 ± 0.292 ^a
Prolactin (ng/ml)	25.91 ± 2.41	23.12 ± 2.39	17.35 ± 2.04 ^a

Each value is Mean ± SEM of 7 animals

^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ control Vs experimental group.

Effect of mercuric chloride on rats.

The immediate effect of LH is to stimulate testosterone synthesis (Rommerts *et al.*, 1983), but it is also believed to be involved in the early development of Leydig cell function (Chase *et al.*, 1982). From this study it is evident that the reduced level of LH due to mercuric chloride treatment might have blocked the conversion of cholesterol into pregnenolone and further pathways leading to testosterone synthesis. Since cholesterol being the precursor for steroidogenic pathway, the reduced testosterone observed in this study after the administration of mercuric chloride may be attributed to the decrease in cholesterol synthesis. Previous study in our laboratory has shown a decreased level of testicular cholesterol by mercuric chloride treatment (Arunadevy *et al.*, 1999).

The data on serum FSH also suggest the inhibitory effect of mercuric chloride. FSH is essential for the spermatogonial differentiation and for the final stages of spermiogenesis (Purvinen, 1982). It has been established that both FSH and testosterone are essential for spermiogenesis in rats (Huang *et al.*, 1991). The final stages of spermatogonial differentiation from intermediate spermatogonia to leptotene primary spermatocyte also require FSH (Means *et al.*, 1976). Therefore, the observed decrease in serum FSH in rats treated with high dose mercuric chloride may affect spermatogenic processes. The decreased serum FSH may also be attributed for the active conversion of available testosterone into estradiol, which in turn might have inhibited FSH release. However, additional information from *in vivo* and *in vitro* studies on the response of the pituitary to gonadotropin releasing hormone stimulating hypothalamic and pituitary response to androgens and estrogens may enlighten this aspect.

From the present study, it is evident that mercuric chloride treatment leads to the impairment in the testosterone production in the testis. This impairment could be due to the reduction in the number of LH binding sites in Leydig cells, as reported earlier in the testicular homogenates of lead treated rats (Kempinas *et al.*, 1990; Thorex-Manley *et al.*, 1997). This impairment could also be due to a decrease in the biological efficiency of LH, and/or to

a failure in steroidogenesis. The reduced testosterone observed due to mercuric chloride treatment may also suggest the impairment of some key enzymes of androgen biosynthetic pathway. The reduction in PRL may be attributed to the possible decrease in the number of LH binding sites in Leydig cells, as it has been shown that PRL has a direct role to play in the regulation of the LH binding sites in testis (Morris and Saxena, 1980).

Thus, from the present investigation, it is clear that mercuric chloride administration alters the hormonal milieu in rats, which may have some adverse effects in the reproductive processes.

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Observations on the histological alterations in various tissues of EUS affected fish, *Channa striatus* (Bloch)

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Abstract : Histopathological investigations have been made on the skin, liver, kidney and intestine of (EUS) affected fish, *Channa striatus* and following anomalies have been observed. Varying degree of degeneration has been observed in the epidermis, dermis, hypodermis and underlying musculature. In all the cases, the skin lost the scales and epidermis completely at the site of infection. The dermis alongwith hypodermis showed the signs of necrosis. Necrosis also took place in subcutaneous layer underlying the hypodermis. Necrotization and formation of granulomas can clearly be seen in circular and longitudinal muscle layers.

The liver exhibited the loosening of tissue and distension in cell bodies. While in case of kidney, shrinkage took place in all the components. Similarly, the intestinal villi got necrotised alongwith their constituent elements.

Key words : Histopathology, Epizootic ulcerative syndrome (EUS), *Channa striatus*.

Introduction

In India, the EUS has assumed the shape of epizootic proportion and has taken heavy toll of millions of fry, fingerlings and adult fishes. The first outbreaks of EUS were reported from India in the month of May 1988 from North eastern region. Thereafter, it spread to Northern and Southern states of the country (Das, 1988; Jhingran and Das, 1990; Pal and Pradhan, 1990; Dastidar and Chakraborty, 1992; Das and Das, 1993; Mohan and Shankar, 1995; Qureshi, *et al.*, 1995, 1998, 1999, 2000 and Mastan and Qureshi; 2001, 2001, 2001). EUS cause severe ulceration in almost all species of fresh as well as estuarine fishes resulting in mass mortality. The present paper reports the histological alterations taken place in various tissues of EUS affected *Channa striatus*.

Materials and Methods

Incidences of EUS were recorded during the winter months of 1997, 1998 and 1999-2000 from almost all the water bodies of Bhopal. The EUS affected fishes were collected and brought to the laboratory in living condition and kept in glass aquaria of the size of 90 x 45 x 45 cm filled with clean freshwater. The dead as well as live fishes were examined grossly for lesions and ulcers.

For histological examinations, the infected tissue was taken out and preserved in aqueous Bouin's fluid for 48-72 hours. The tissue was then processed routinely and prepared into paraffin blocks. The blocks of the tissue were cut at 4 μ m thickness and stained with Delafield's Haemato-xylin and Eosin (H-E). Standard histopathological procedures (Roberts, 1989) were followed for histopathological investigations.

Results and Discussion

Histopathological investigations of skin of *Channa striatus* reveals that, varying degree of degeneration has been taken place in the epidermis, dermis, hypodermis and underlying musculature. In all the cases, the skin lost the scales and epidermis completely at the site of infection. The dermis alongwith hypodermis showed the signs of necrosis. Necrosis also took place in sub-cutaneous layer underlying the hypodermis. Longitudinal muscle fibres clearly showed the sign of necrosis. Necrotization and formation of granulomas can clearly be seen in the circular and longitudinal muscle layers (Figs. 1, 2 and 3).

In infected fish, the liver showed the loosening of tissue and distension of the cells. Many

of the distended cells appear to become empty and have lost their nuclei (Fig. 4).

In infected fish, degeneration took place in the renal tubules as well as hematopoietic tissue

because of which shrinkage set in all the components of kidney. Due to shrinkage, the uriniferous tubules became constricted and their internal spaces were greatly reduced. The glomeruli appear smaller in size



Fig. 1 : Microphotograph of a cross section of skin of *C. striatus* showing (arrow) necrotization in epidermis and dermis x 250.



Fig. 2 : Microphotograph of a cross section of skin of *C. striatus* showing (arrow) necrotised muscle fibres x 250.

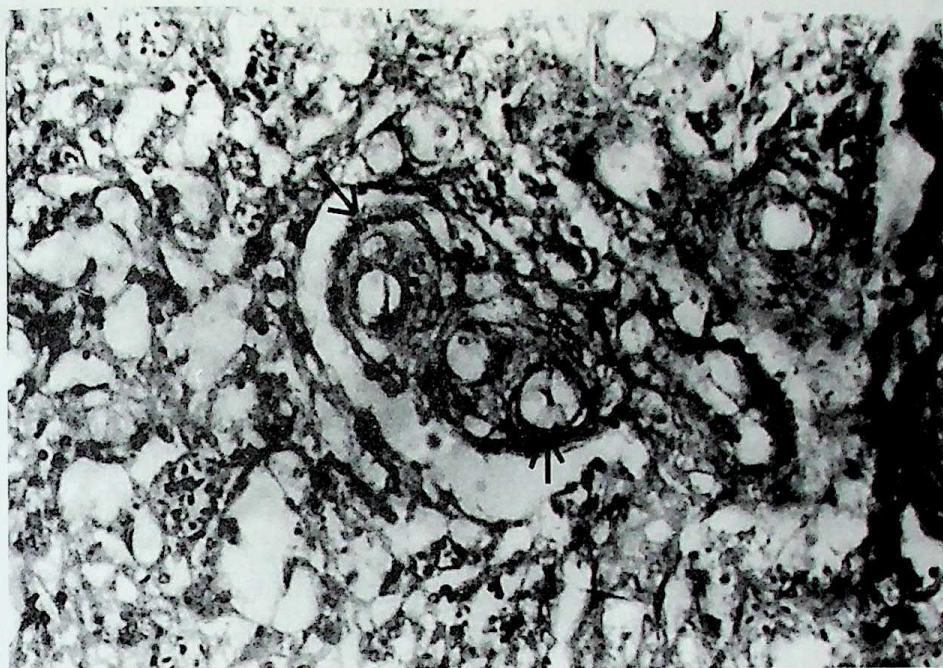


Fig. 3 : Microphotograph of a cross section of skin of *Channa striatus* showing (arrow) several granulomas in muscular layer x 400.

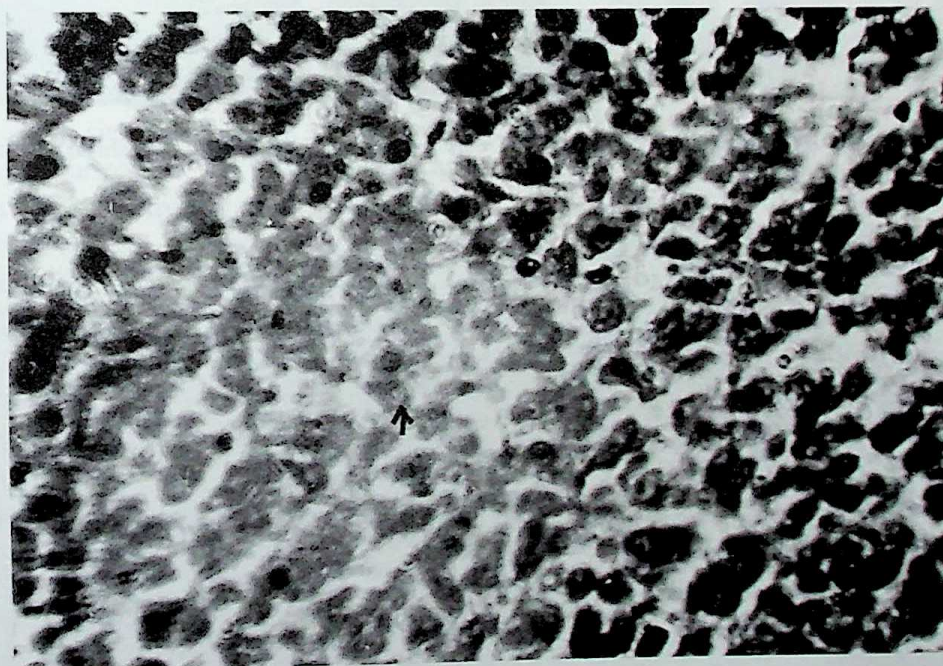


Fig. 4 : Microphotograph of a cross section of liver of *Channa striatus* showing (arrow) distended hepatic cells, many of which have lost their cytoplasm as well as nuclei x 250.

(Fig. 5). In infected fish, necrotization of the intestinal villi alongwith their constituent cellular elements have been observed (Fig. 6).

EUS has not been sufficiently studied histopathologically. However, general histopathological manifestations of the disease are known to

some extent. Some of the early skin lesions were characterized by the presence of epithelial necrosis with surrounding odema, haemorrhages of the underlying dermis and some inflammatory cell infiltrations, often complicated by the presence of parasitic forms (Lilley *et al.*, 1992). Roberts *et al.*

(1989) have observed more severe necrotizing myopathy in Indian major carps. Kumar *et al.* (1991) reported loss of epidermis on the ulcers with characteristic cyst like granuloma formation in

abundant number in dermis, hypodermis and muscle with high degree of inflammation.

The internal organs of the diseased fish usually showed minimal changes (Palisoc, 1990)

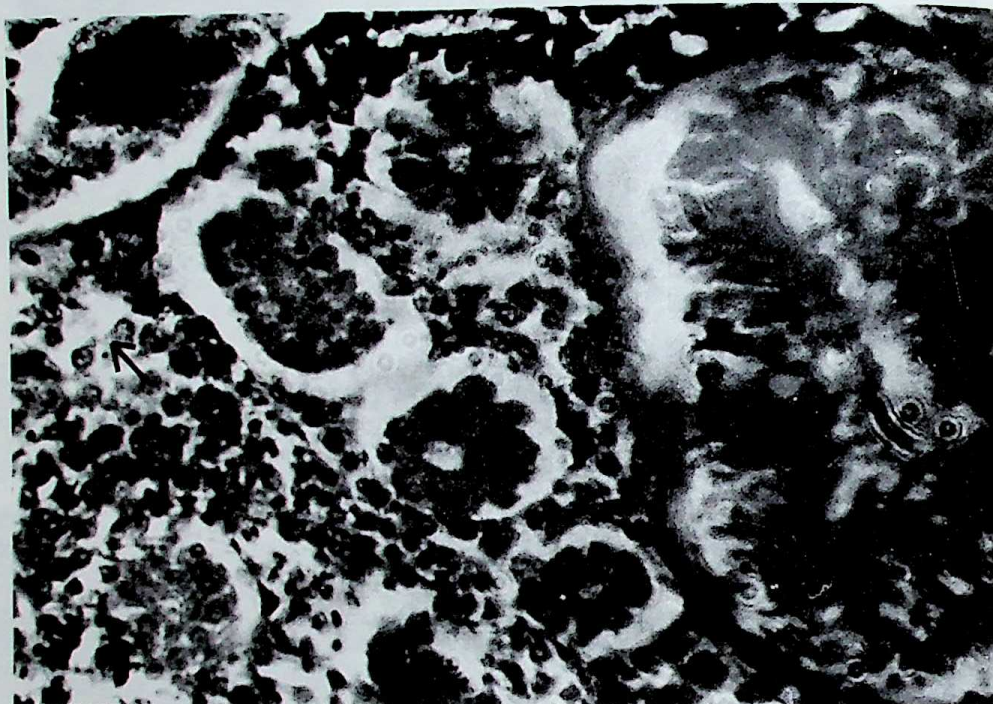


Fig. 5 : Microphotograph of a cross section of kidney of *Channa striatus* showing (arrow) shrinkage in almost in all the components x 250.



Fig. 6 : Microphotograph of a cross section of intestine of *Channa striatus* showing (arrow) necrotization of intestinal villi along with their constituent cellular elements.

Histological variations in tissues of EUS affected fish.

except when invaded by fungi (Lilley *et al.*, 1992), Palisoc (1990) observed minimal changes in kidney of snakehead, increase in white pulp of spleen and mild histopathological changes in heart, liver and gills and none in the stomach and intestine. Chinabut (1990) also observed the degenerative changes in the kidney of spiny eel.

Other changes observed in the liver and spleen include vacuolation (Pal and Pradhan, 1990), congestion of sinusoidal space and vessels with plenty of wandering lymphocytes in liver (Kumar *et al.*, 1991), depletion of white cells with pericapsular odema in spleen (Roberts *et al.*, 1992) necrotic changes in white pulp (Pal and Pradhan, 1990).

In the present study, it has also been observed that varying degree of degeneration took place in the epidermis, dermis hypodermis and underlying musculature. In all the cases, the skin lost the epidermis completely at the site of infection. The dermis along with hypodermis showed the sign of necrosis. Necrosis also took place in subcutaneous layer underlying the hypodermis. Longitudinal muscle fibres clearly showed the sign of necrosis. Necrotization and formation of granulomas can clearly be seen in the circular and longitudinal muscle layers. Infected liver showed the loosening of tissue and distension of the cells. Many of the distended cells lost their nuclei. Degeneration also took place in the renal tubules as well as hematopoietic tissue as a result of which shrinkage set in all the components of kidney. In infected fish, necrotization of the intestinal villi along with their constituent cellular elements has been observed. These findings are in agreement with the observations of Kumar *et al.* (1991), Roberts *et al.* (1993), Mohan and Shankar (1995) Chinabut *et al.* (1995) and Vishwanath *et al.* (1997a, 1997b, 1998).

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Effects of lead on exploratory behavior and running speed in the shrew, *Blarina brevicauda* (Insectivora)

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Abstract : These studies were conducted to assess the effects of lead toxicity on exploratory behavior and running speed in the short-tailed shrew, *Blarina brevicauda*. Shrews from the experimental group received 25 mg/kg/day of lead acetate in their drinking water for a period of 90 days. Control subjects received sodium acetate. Exploratory behavior was determined using a computerized activity chamber where movements of test subjects broke infrared beams projected onto the floor of the apparatus. Time spent (sec) in exploration was recorded over eight 6-min intervals. Running speed (km/hr) was measured in a microprocessor-controlled rectangular racetrack fitted with photocell timers. With respect to time spent in exploration, there were significant differences between lead-exposed (20.5-23.9 sec per 6-min testing session) and control subjects (6.8-8.1 sec) after the sixth testing interval in the activity chamber. With respect to maximal running speed, control subjects ran significantly faster (mean : 14.8 km/hr) than their lead-exposed counterparts (5.83 km/hr). Lead-exposed animals exhibited hyperactivity and increased random locomotor movements. They would frequently bump into the walls and their movements were more random. Controls typically ran along the racetrack in a straight line. These results represent the first data for the effects of lead exposure on exploratory behavior and running speed for shrews.

Key words : *Blarina brevicauda*, Exploratory behavior, Lead exposure, Running speed.

Introduction

Lead is a well known central nervous system (CNS) toxicant and is a common contaminant of air and water. Lead compounds that accumulate in mammals have been shown to adversely affect various physiological parameters including the development of the CNS (Gilbert, 1997), neurotransmitter synthesis and function (Meredith *et al.* 1988), reproduction (Goyer, 1991), kidney function (Jones *et al.*, 1994), growth rates (Pankakoski *et al.*, 1992), and metabolic processes (Ma, 1991). Previous studies, conducted primarily on rodents and primates, have also shown that lead can affect behavior as well, including locomotor activities (Hall *et al.*, 1990), learning and memory (Brown *et al.*, 1971; Snowdon, 1973; Altmann *et al.*, 1993), and exploratory behavior (Dolinsky *et al.*, 1981; Riess and Needleman, 1992). Little information is available on the effects of lead on behavioral parameters in shrews and other insectivores.

Shrews have been identified as indicator species for the analysis of heavy metal accumulation

in terrestrial ecosystems (Pankakoski *et al.*, 1994). Shrews and other small mammals can exhibit high population densities and thus provide sample sizes adequate for toxicological studies. Because of the greater consumption rates associated with their high rates of metabolism, shrews can accumulate significant levels of toxic compounds. In addition, the types of prey items that they typically feed on, such as earthworms and insects, readily accumulate heavy metals in their body tissues as well (Goldsmith and Scanlon, 1977; Ma, 1989). Although there have been numerous studies on the levels of heavy metals associated with body tissues in shrews (see review by Pankakoski *et al.*, 1994), little data are available on the effects of these metals on shrew behavior. The present study was undertaken in order to evaluate the effects of lead accumulation on exploratory behavior and running speed in the short-tailed shrew, *Blarina brevicauda*. Exploratory behavior is an important parameter because it is associated with search paths and foraging efficiency (Punzo, 2003), while the ability of cursorial mammals to escape potential predators can be markedly affected by running speed (Djawdan and Garland, 1988).

Materials and Methods

The animals used in these experiments were adult males and females (5 months old; 18-20 g) obtained from a captive breeding colony established in 1997 from adults collected in northern Hillsborough Co., FL. Animals were maintained in plastic rodent cages in a room where temperature, humidity, and photoperiod were controlled (21-22°C, 60-65% RH, 10L : 14D). They were provided with water *ad libitum*, and fed on a diet consisting of commercial cat chow (Alston Purina, St. Louis, MO) and chopped beef heart. In order to determine the effects (if any) of lead on behavior, a group of 100 two-month old males and females were randomly selected from the laboratory colony and exposed to a 25 mg / kg / day concentration of lead acetate solution administered in their purified, distilled drinking water (Brown, 1975) for a period of 90 days (lead-exposed group). Preliminary studies showed that while these doses did not result in a significant decrease in survivorship, body weight, or growth rates in this species, it resulted in the accumulation of lead in various body tissues ranging from 27-33 ~g / g dry weight. These levels have been reported for other species of shrews from urban locations (Goldsmith and Scanlon, 1977; Pankakoski *et al.*, 1994). Another group (N = 50) was administered the same concentration of sodium acetate (Sigma Chemical Co., St. Louis, Missouri; S-9513; 0.005% insoluble matter) and served as the control group. These animals served as the pool of subjects for subsequent behavioral experiments.

Twenty-five males and 32 females were randomly selected from the lead-exposed shrews for experiments on exploratory behavior. Controls consisted of 11 males and females. Exploratory behavior was determined using a computerized, automated, and rectilinear vinyl activity chamber (61 x 31 x 48 cm) according to the method described by Hall *et al.* (1990) for meadow voles. To summarize, the floor of the chamber contained 4 exploratory holes, one at each corner of the chamber. Each hole was provided with an infrared beam emanating from the top of the chamber and focused at its entrance, to detect when an animal investigated it, and for how long. The total duration of beam breaks was used as

a measure of exploratory behavior. Animals were tested individually for 48 min in the activity chamber. A Macintosh G3 computer monitored all events and recorded the duration of beam breaks. For analysis, the 48-min test period was divided into eight 6-min intervals. A Wilcoxon two-sample test, performed for each sex, was used to test for significant differences between water-exposed and control shrews in each of the eight time intervals as described by Hall *et al.* (1990). For experiments on the effects of lead exposure on running speed, 14 adult male shrews were randomly selected from the control and lead-exposed groups. Shrews were deprived of food for 8 hr prior to testing. A detailed description of the apparatus and protocol used to determine running speeds can be found in Djawdan and Garland (1988). To summarize, maximal running speeds were determined by timing shrews as they ran along a microprocessor-controlled rectangular racetrack (2 m x 10 cm x 10 cm) fitted with photocell-timers. Beginning at the start-end of the track, nine sets of vertically-aligned photocells were placed at 0.1 m intervals along the base of the floor to allow for the determination of running speed. The floor was covered with artificial turf to provide for traction. The end of the track contained a darkened box that provided a refuge into which the shrews ran. Running speeds were expressed in km/hr. To allow the animals to habituate to test conditions, each shrew was chased slowly back and forth along the track 4-5 times before actual testing began (Djawdan and Garland, 1988). At the start of each trial, one shrew was placed at the start-end of the track and coaxed to run by gently prodding it with a padded wooden dowel. After each trial the animal was returned to the start area. Multiple trials (4-7) were run for each shrew until no further increase in speed was observed for subsequent trials. The fastest time was defined as maximal running speed (Huey, 1982). The mean maximal running speeds for both groups were analyzed using a t-test (Djawdan and Garland, 1988).

Results and Discussion

The effects of lead exposure on exploratory behavior over 8 time intervals are shown in Table 1.

Table - 1 : Exploratory behavior of lead-exposed (25 mg/kg/day lead acetate, for 90 days) and control (sodium acetate) male and female shrews (*Blarina brevicauda*). Behaviors were quantified as time spent in exploration (in sec) over eight 6-min intervals. Data expressed as means \pm S.E.* Values significantly different than controls, $P < 0.01$.

Time interval	Number :	Exploration time (sec)			
		Lead-exposed		Controls	
		Males (25)	Females (32)	Males (11)	Females (11)
1		49.2 \pm 6.1	47.2 \pm 5.1	53.4 \pm 7.4	52.2 \pm 6.8
2		41.4 \pm 5.3	43.8 \pm 5.2	43.1 \pm 6.6	48.9 \pm 7.1
3		43.6 \pm 5.9	40.8 \pm 4.7	45.3 \pm 6.9	41.8 \pm 8.6
4		38.7 \pm 2.2	36.5 \pm 3.1	41.3 \pm 6.3	43.2 \pm 7.1
5		23.6 \pm 3.8	25.1 \pm 4.5	24.3 \pm 3.9	22.6 \pm 2.1
6		21.7 \pm 3.3*	23.5 \pm 2.8*	7.7 \pm 0.5	9.3 \pm 1.2
7		20.5 \pm 2.8*	22.4 \pm 2.2*	8.1 \pm 1.1	6.8 \pm 0.8
8		22.6 \pm 4.4*	23.9 \pm 2.8*	6.9 \pm 0.4	7.8 \pm 1.1

There were no significant differences between males and females in the lead-exposed or control groups over the entire test period ($p > 0.50$). Males and females from both groups spent similar amounts of time in exploration over the first 5 time intervals. However, by the sixth time interval, lead-exposed shrews spent significantly more time in exploration than controls ($s = 163.7$, $p < 0.01$). This increase in exploration time was also observed for the seventh and eighth time intervals as well. Hyperactivity has been identified as a symptom of exposure to heavy metals including cadmium and lead (Tilson *et al.*, 1982; Ruppert *et al.*, 1985; Rice, 1993). It has also been demonstrated that exposure to neurotoxins can result in a change in behavior to novel environments (Riess and Needleman, 1992; Gilbert, 1997). The increased levels of exploration by these shrews towards the end of the 48-min test period is in general agreement with results on exposure to several heavy metals on locomotor activity and exploratory behavior in other species of mammals (Ruppert *et al.*, 1985; Riess and Needleman, 1992), and may be due to lead-induced hyperactivity. Hyperactivity may, in turn, increase random locomotor activities and potential exposure to predators.

With respect to maximal running speed, control male shrews ran significantly faster (mean : 14.8 km/hr \pm 2.4 S.E.; range : 12.9-17.2 km/hr) than

their lead-exposed counterparts (mean : 5.1 km/hr \pm 1.2; range : 3.8-7.8) ($t = 5.83$, $P < 0.01$). Although lead-exposed shrews exhibited a heightened level of general locomotor activity, they would frequently move from side to side in the racetrack, bumping into the walls, and their movements appeared to be more random. The control animals, on the other hand, typically ran along the track in a straight line from the start area to the end of the runway, thereby achieving faster running speeds. Reduced locomotor performance may make shrews more vulnerable to predation by ground-hunting predators and by avian predators to a lesser extent. Shrew remains have been found in the stomach contents and fecal material of rats, weasels, foxes, feral house cats, owls, hawks, and snakes (Churchfield, 1990). To our knowledge, these experiments are the first to report on the effects of lead exposure on exploratory behavior and running speed in shrews. The results suggest factor in shrew mortality whenever these animals are associated with microhabitats contaminated with lead compounds.

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Evaluating the seasonal changes of water quality of the Değirmendere and Galyan Rivers (Trabzon, Turkey)

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Abstract : The Değirmendere and Galyan (Değirmendere tributary) Rivers that discharge their water into the Black Sea are important watersheds in the northeastern part of Turkey. Water quality parameters were sampled from 1997 through 2001 for each year at five sites (three for Galyan, two for Değirmendere) along 29 and 42 km gradients, respectively covering all seasons. Surface water was collected from the sites and analyzed for temperature, total alkaline (MAL), total dissolved solids (TDS), dissolved oxygen (DO), pH, conductivity (EC), nitrate (NO₃), nitrite (NO₂), total hardness (TH), phenolphthalein alkalinities (PAL) and organic matter (PV). Seasonal changes of water quality were analyzed statistically for both Rivers and evaluated according to the TS 266, EU and WHO standards. The analysis of variance results showed that Ca, Mg, MAL, NO₃, pH, TDS and TH parameters of the Değirmendere River and Ca, DO, EC, MAL, NO₃, pH and TH parameters of the Galyan River showed seasonal differences ($p < 0.05$). The maximum values of the water pollution parameters for the two Rivers were below the threshold values throughout the study period. When both Rivers were compared, the mean values of the pollution parameters of the Değirmendere River were higher than those of the Galyan River and very close the limits. The results indicate that both Rivers can be used for the production of potable water during all seasons but only with an advanced treatment in the Değirmendere and a moderate treatment in the Galyan River, and for indirect and non-contact recreational activities.

Key words : Water quality parameters, Değirmendere River, Galyan River, Water pollution.

Introduction

Water has been one of the most important strategic natural resources for mankind throughout the history. However, the world's water resources are under pressure and in danger because of potential pollution and contamination risks due to overuse and misuse of the resources. People strive to sustain their lives under inappropriate environmental conditions. Streams are the clean water resources among all water resources. Clean water resources are more prone to pollution compared with spring water. In developing countries such as Turkey, 95% of the used water is not distilled or purified before they are released to surface water. As a result water pollution occurs.

Trabzon province, located within the eastern part of Turkey, is an ancient residential area. The Değirmendere River, passing through Trabzon and draining into the Black Sea, is the most important watershed and a crucial source for municipal drinking water supply as well as for heavy and light industries, and water dilution. The watershed covers

104,172 ha of land area and is home for 110 villages with a population over 43,000 people. The Galyan River meets the Değirmendere River at a conjunction on the 17th km of Trabzon-Macka highway. The Galyan watershed covers 18,905 ha of land area (Altun *et al.*, 2001). Ten villages with 5,244 people are situated within the watershed (Anonymous, 2000a).

The Değirmendere and the Galyan Rivers are easily polluted and contaminated because of natural and anthropogenic disturbances. The water of the Rivers are polluted due mainly to the discharged waste water from residential areas, leakage from gas stations and sewage canals, solid wastes, detergents, automobile oil wastes, fishing facilities and the use of agricultural pesticides on farmlands along the watersheds. Such irresponsible situation exacerbates the quality of the water resources and limits or even prevents the use of the water for various purposes as they threaten the human health and aquatic life seriously.

The water pollution resulting from the sources mentioned above reached a level such that water from the Değirmendere River started to threaten even the health of people. Furthermore, increasing cost of purifying the polluted water for daily drinking called for alternative sources of water supply. As a result, Atasu Dam was initiated 7 km east to the junction of the Değirmendere and Galyan Rivers by the 22nd Regional Directorate of State Water Relations to provide clean water according to potable water standards for the people of Trabzon. The first objective of the project was to provide clean and quality water with an affordable cost to the people of Trabzon, and the second, to generate hydroelectric energy from the dam.

The main objective of this study is to assess the seasonal changes of water quality in Değirmendere and Galyan Rivers from 1997 to 2001. Water samples were taken periodically from both Rivers over four years and analyzed in the lab.

The data from the samples were statistically analysed and the results were discussed.

Materials and Methods

Study area : The research area, located at $39^{\circ} 39' - 39^{\circ} 45' E$ and $40^{\circ} 45' - 40^{\circ} 52' N$, covers Değirmendere and Galyan watersheds as well as Simsirli River watershed, a tributary of the Galyan River (Fig. 1).

Both The Değirmendere and the Galyan watersheds are strongly influenced by the rain bearing air masses brought over by the northerly winds coming from the Black Sea. These air masses drop their loads over the region in the form of snow during January and February and generally rain in other months. Snow masses over the mountains with high elevation (1500 m-2100 m) begin to melt around the middle of March due to increasing air temperatures and, as a result, surface water levels increase.

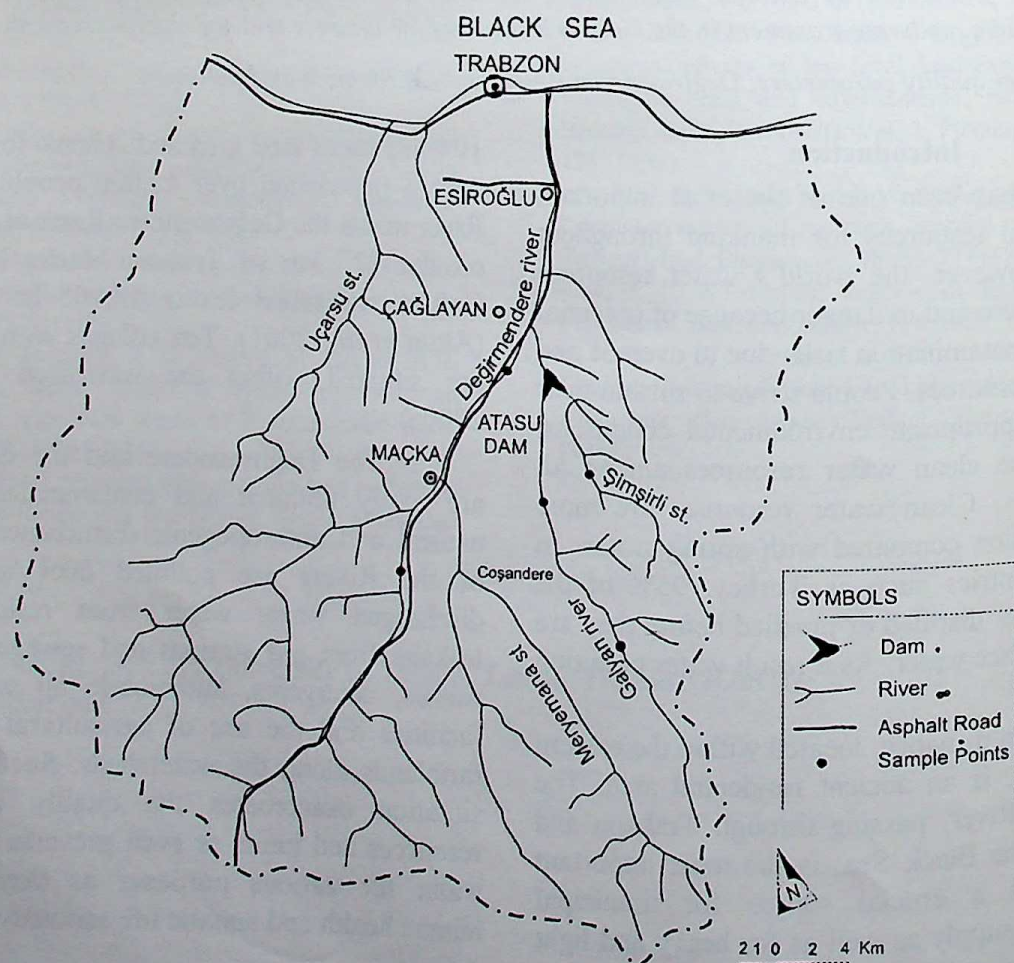


Fig. 1 : The research area with the location of the sample plots.

These differences were found for Ca, MAL, NO₃ and TH parameters in the Değirmendere River at $p=0.01$, 0.01, 0.001 and 0.004 respectively. The Ca, MAL, NO₃ and TH parameters reached their highest values in winter. Likewise, the Mg, pH and TDS parameters showed similar changes over seasons. Conversely, though, the Mg, pH and TDS parameters reached their highest values in summer. The Ca, DO and NO₃ parameters of the Galyan River obtained their highest values in winter, EC, MAL and pH parameters in summer, and PAL and TH parameters in fall.

The other parameters determined in each River and shown in Table 1 are discussed as following. The rate of nitrate (NO₃) in the Galyan River was found to be much higher than that in the Değirmendere River. This can be explained in multifold. The Galyan watershed is relatively narrower and steeper. Agricultural field crops such as hazelnut, potatoes and corn starts immediately near the Riverbanks with slopes well over 20%, indicating that much of the land is unsuitable for agriculture (Altun *et al.*, 2001). Thus, farmers often use nitrogen-based fertilizers [(NH₄)₂SO₄, HNO₃] to increase the production capacity of the fields. Consequently, the fertilizers are easily washed and transported into both surface and ground water in short time helping nitrate (NO₃) to accumulate easily.

Electrical conductivity of potable water may reach, at maximum, 2000 $\mu\text{mhos/cm}$ (Altınyar *et al.*, 1994). The electrical conductivity values were $193.443 \pm 85.044 \mu\text{mhos/cm}$ in The Değirmendere River and $143.777 \pm 65.625 \mu\text{mhos/cm}$ in the Galyan River. Both values are within the potable water standards.

The amount of chloride ions is an indicator of healthy potable water. In various types of drinking water, the amount of chloride ions does not exceed 30 mg/l (Egemen and Sunlu, 1996). Kuruma *et al.* (2002) found the average values of chloride to be 11.4 mg/l. The chloride values of this study are found to be $4.716 \pm 2.866 \text{ mg/l}$ in Değirmendere River and $2.755 \pm 1.893 \text{ mg/l}$ in Galyan River. The value in Değirmendere River is within the potable water standards and can be classified within the first

class drinking water group according to terrestrial water quality criteria.

The average ammoniac (NH₃) values are found to be $0.309 \pm 0.618 \text{ mg/l}$ in Değirmendere River and $0.115 \pm 0.127 \text{ mg/l}$ in Galyan River. Based on these values, the water of the Değirmendere and Galyan Rivers can be classified as 2nd and 1st class drinking water, respectively, according to the drinking water standards,

The main sources of nitrate that showed significant seasonal differences in Değirmendere water are the organic matters, N mixed fertilizers and some other minerals found in nature (Egemen and Sunlu, 1996). In addition, nitrite, which did not show a significant difference, is converted to nitrate through oxidation. As a result, nitrite in aerobic environment can exist in natural water in a short period of time (Giritlioğlu, 1975).

Furthermore, inorganic nitrates may be reduced in an environment with an inadequate amount of oxygen to produce nitrites and ammoniac. Nitrite is produced during the oxidation reaction of ammoniac to nitrate and in the reduction of nitrate. Nitrites in acidic environments can engage into a reaction with secondary amines, and nitrous amines having carcinogen effect are then produced. Given this fact, the nitrite values cannot exceed 0.1 mg/l in potable water (Özçelik, *et al.* 2001). The maximum nitrite values are found to be 0.023 mg/l in fall in Değirmendere water samples and 0.014 mg/l in winter in the Galyan water samples. This result indicates that nitrite is highly oxidized with oxygen in the Galyan River. This is of a great importance for the quality of drinking water.

Nitrate concentration should be 50 mg/l at most according to water quality standards. Nitrate level in Değirmendere River was determined to be 1.749 mg/l in winter and 1.810 mg/l in winter in the Galyan River. These values are well below the suggested level of drinking water standards (according to Institute of Turkish Water Standard (TS 266), European Commission (EU) and World Health Organization (WHO)).

The maximum level of Ca was 82.122 mg/l in the Değirmendere River in winter season while it

was 67.083 mg/l in the Galyan River in the same season. These values are within the suggested level

of drinking water standards according to TS 266, EU and WHO standards.

Table - 1 : Seasonal variation of some water quality parameters in Değirmendere and Galyan Rivers.

Parameters	Season	Değirmendere River					Galyan River				
		N**	Mean (std. dev.)	G***	F	Significant level	N**	Mean (std. dev.)	G***	F	Significant level
Calcium (mg/l)	1	14	82,12± 83,06	c			21	67,08 ± 81,75	c		
	2	10	16,73± 9,75	a	4,26	0,009	14	14,16 ± 5,11	a	4,81	0,004
	3	18	34,29 ± 17,45	ab			28	28,28± 12,41	a		
	4	8	64,22± 50,53	bc			11	31,31± 11,79	a		
Chloride (mg/l)	1	14	4,63± 3,46	a			21	3,10± 2,00	a		
	2	9	3,96± 2,47	a	0,53		13	3,16± 1,00	a	0,98	0,408
	3	19	4,72± 2,82	a		0,66	29	2,31± 2,03	a		
	4	8	5,72± 2,39	a			11	2,08 ± 2,07	a		
Dissolved oxygen (%)	1	9	33,01± 63,41	a			14	54,84± 75,68	b		
	2	6	19,53± 17,18	a	0,65		9	19,57± 16,74	ab	3,20	0,033
	3	12	14,52± 18,50	a		0,58	18	12,77 ± 19,66	a		
	4	4	5,62± 3,00	a			7	5,25± 2,35	a		
Conductivity (EC) (µmhos/cm)	1	13	190,11± 89,97	a			20	129,83± 70,68	ab		
	2	10	141,60± 25,02	a	1,86		14	129,83± 26,43	a	3,69	0,016
	3	20	212,01± 86,03	a		0,148	29	110,07± 60,67	b		
	4	8	217,25± 106,7	a			11	169,10± 80,26	b		
Potassium (mg/l)	1	12	1,20± 0,71	a			19	0,82 ± 0,47	a		
	2	9	0,75± 0,36	a	0,55		12	0,83± 0,25	a	0,53	0,663
	3	20	1,95± 3,79	a		0,65	29	0,97± 0,66	a		
	4	8	1,36± 0,61	a			11	0,8± 0,37	a		
Total alkaline MAL (mg/l)	1	14	125,96± 53,14	b			21	68,09± 0,26	ab		
	2	9	62,59± 41,31	a	3,56		13	49,09± 0,66	a	4,41	0,007
	3	18	119,17± 48,95	b		0,021	28	96,79± 0,37	b		
	4	8	118,75± 50,69	b			11	91,82± 19,21	b		
Magnesium (mg/l)	1	14	9,58± 4,66	b			20	6,24± 27,52	a		
	2	10	4,16± 2,68	a	3,56		14	5,03± 59,09	a	0,66	0,579
	3	20	7,31± 4,90	ab		0,021	29	5,51± 42,98	a		
	4	8	4,97± 1,27	a			11	4,93± 2,09	a		
Sodium (mg/l)	1	12	6,13± 4,64	b			19	3,39± 4,39	a		
	2	9	2,78± 1,32	a	2,23		12	2,72± 3,11	a	2,23	0,093
	3	20	5,61± 2,81	ab		0,097	29	3,85± 1,44	a		
	4	8	4,71± 2,76	ab			11	2,68± 2,97	a		
Ammoniac (NH ₃) (mg/l)	1	14	0,43± 1,05	a			21	0,10± 1,77	ab		
	2	10	0,20± 0,21	a	0,28		14	0,14± 0,53	b	1,79	0,158
	3	18	0,28± 0,34	a		0,84	26	0,14± 0,82	b		
	4	6	0,29± 0,43	a			10	0,05± 1,35	a		
Nitrite (mg/l)	1	14	0,01± 0,01	a			21	0,01± 0,16	a		
	2	10	0,01± 0,01	a	1,06		14	0,006± 0,14	a	0,38	0,766
	3	18	0,01± 0,02	a		0,374	26	0,006± 0,10	a		
	4	8	0,02± 0,03	a			11	0,007± 0,89	a		

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Nitrate (mg/l)	1	12	1,75±0,71	b	11,76	0,00006	20	1,81±0,67	b	5,32	0,002
	2	10	0,79±0,42	a			14	1,04 ± 0,47	a		
	3	18	0,80±0,27	a			26	1,18 ± 0,43	a		
	4	8	1,11±0,41	a			11	1,17 ± 0,00	a		
Phenolphthalein alkalinity (PAL) (mg/l)	1	14	0,00±0,00	a	1,58	0,206	18	0,00 ± 0,66	a	2,66	0,056
	2	8	0,00±0,00	a			12	2,50 ± 0,07	a		
	3	18	7,22±16,74	a			25	2,00 ± 25,39	a		
	4	8	8,75±16,42	a			9	12,22 ± 0,71	b		
pH	1	14	7,40±0,68	a	3,51	0,022	21	7,44 ± 0,64	a	3,15	0,030
	2	10	7,79±0,48	ab			14	7,72 ± 0,21	ab		
	3	20	7,96±0,28	b			29	7,87 ± 0,36	b		
	4	8	7,69±0,60	ab			11	7,57 ± 0,09	ab		
Organic matter (PV) (mg/l)	1	11	6,00±9,36	a	1,78	0,16	17	6,83 ± 1,85	a	2,30	0,087
	2	9	1,97±1,92	a			11	2,65 ± 1,92	a		
	3	17	1,93±1,91	a			23	2,21 ± 1,57	a		
	4	4	1,31±1,20	a			6	1,40 ± 12,64	a		
Suspended solids (mg/l)	1	4	195,00±52,60	a	1,02	0,413	10	87,00 ± 95,08	a	0,47	0,704
	2	4	200,00±318,0	a			8	98,75 ± 82,32	a		
	3	8	73,75±65,23	a			17	74,71 ± 26,30	a		
	4	2	225,00±63,64	a			4	42,50 ± 33,07	a		
Total dissolved solids (mg/l)	1	4	175,00±19,15	a	17,62	0,0001	10	144,00 ± 40,33	a	0,49	0,692
	2	4	77,50±20,62	b			8	123,75 ± 49,31	a		
	3	8	151,25±15,53	bc			17	137,65 ± 16,33	a		
	4	2	120,00±42,43	c			4	150,00 ± 28,84	a		
Total hardness (mg/l)	1	14	155,81±60,95	a	5,01	0,004	21	89,70 ± 16,29	b	3,63	0,017
	2	9	79,69±19,70	a			14	64,23 ± 38,97	a		
	3	19	123,58±39,79	a			29	97,55 ± 36,24	b		
	4	8	130,43±50,82	a			11	97,98 ± 82,39	b		

* Season : 1. Winter, 2. Spring, 3. Summer, 4. Autumn

** N: Number of samples

*** G : Different letters show different groups according to Duncan's multiple test.

Total hardness is formed with the weathering of alkaline minerals in soil and rocks and is quantified to be 7.5-17.5⁰F in productive waters (Oruç, 1972). The average total hardness of the water in the Değirmendere River was recorded to be 125.800 ± 51.506 mg/l and 89.194 ± 34.357 mg/l in the Galyan River. These values of both Rivers were above the standards and are classified as very hard water class.

The total hardness values of waters for both Değirmendere and Galyan Rivers differ over seasons. While the highest values of 155.814 mg/l were recorded in winter for Değirmendere, they were of 97.977 mg/l for Galyan River in fall. As known, Mg is one of the other parameters to affect the total hardness of water. In our study, the Mg values of water in Değirmendere River showed significant seasonal differences. Table 1 clearly depicts that the

highest values of Mg (9.575 mg/l) was in winter. The fact that both Ca and Mg cations had highest values in winter caused the total hardness to be high in winter. That both Ca and Mg cations showed their highest values in winter is mainly due to washing off the Ca and Mg cations which are found either in the soils formed by parent rocks or in fertilizer often used in agricultural, through precipitation or with rain and reaching to the water in the River.

For aquatic life, the optimal range of pH, an important factor for biological and chemical systems of natural water, is between 8.5 and 9.0 (Soylu, 1984). However, according to the potable water standards the pH values of drinking water should be between 6.5 and 8.5 (Hölting 1984; Moson and Moore 1985). Şimşek *et al.* (2001) found the pH values between 6.9 and 8.0. In this study, however, the highest pH value of 7.9 was observed in

Değirmendere River and 7.8 in the Galyan River in summer. The pH values of both Rivers indicate alkaline character. The highest pH values being in the summer is meaningful and significant. Less amount of rain in summer reduces the volume of the River flow, which, in turn, reduces the organic matter carried in the water. Low concentration of dissolved products in the water indicates an acidic state and might not have affected the changes in pH values. The Değirmendere and the Galyan River water can be classified as having the first class water quality according to the inland terrestrial water standards. Furthermore, the water in both Rivers is suited to or aligned with the TS 266, EU and WHO criteria.

The amount of total dissolved solids in Değirmendere River showed highly significant differences over seasons, reaching a maximum of 175 mg/l in winter. A number of factors such as the size of watershed, size of the degraded forested area, overgrazed meadows; large eroded areas and dense residence areas would affect these differences. The high amount of rainfall in the winter easily carries materials through erosion on the degraded forest areas, overgrazed areas and highly populated residential areas, contributing to the increase of the total amount of dissolved solids in the River. The amount of total dissolved solids in Galyan River, however, showed no significant differences over seasons, reaching maximum of 150 mg/l in fall. This could be explained by the fact that the Galyan watershed area is not mismanaged as in the case of Değirmendere watershed.

The electrical conductivity values in Galyan River showed highly significant differences over seasons, reaching a maximum of 169.103 $\mu\text{mhos/cm}$ in summer. The electrical conductivity values in Değirmendere River, however, showed no significant differences over seasons, reaching a maximum of 217.25 $\mu\text{mhos/cm}$ in fall. The maximum values in both watersheds found in fall and summer are due mainly to the increased amount of ion concentration generated because of River vaporization in those seasons.

The dissolved oxygen (DO) in Galyan River showed highly significant differences over seasons

and reached a maximum value of 54.83% in winter - a value that is well below the required European Union standard value of 75%. The dissolved oxygen (DO) in Değirmendere River showed no significant differences over seasons, and had a maximum value of 33% in winter. These DO values in both watersheds indicate the presence of an advanced water pollution resulting from organic matter. In fact, the maximum organic matter (PV) values that determine organic pollution were determined in winter. These values were recorded as 6.83 mg O_2/l in Galyan River and 6.00 mg O_2/l in Değirmendere River. Polat *et al.* (2001) determined the PV values to be between 0.60-1.6 mg O_2/l . According to TS 266 (1997), the PV values of drinking water should be below 3.5 mg O_2/l level. It is well known that the increased amount of organic matter in the surface water is an important indicator of water pollution. The maximum values of organic matter recorded in both Rivers in winter results in a decrease in the amount of dissolved oxygen in water. When the level of organic matter is evaluated according to the Inland Terrestrial Water Sources, the surface water of both Rivers exceeds the threshold values.

Furthermore, the physical and the chemical parameters measured from the surface water samples in Galyan and Değirmendere Rivers area evaluated according to the criteria set by TS 266 (1997), EU (1980) and WHO (1993) standards. The nitrate values (0.11) of the Galyan River show suitable condition while the nitrate values (0.30) of the Değirmendere River exceed the threshold values.

On the other hand, the PAL values of surface water in both Rivers exceed the threshold values set by TS 266 (1997), EU (1980) and WHO (1993). Other parameter values are within the standard values.

When the surface water pollution parameters are evaluated together, Ca, Cl, EC, K, MAL, Mg, Na, NH_3 , NO_2 , PAL, pH and TH parameters show higher levels in Değirmendere River and DO, NO_3 , PV and TDS parameters in Galyan River. The maximum values of the water pollution parameters in both Rivers over the seasons of the five-year study period were below the threshold values. When both Rivers were compared, however, the mean values of

the pollution parameters of the Değirmendere River were higher than those of the Galyan River and very close the limits. The results indicate that both Rivers can be used for the production of potable water during all seasons but only with an advanced treatment in the Değirmendere River and a moderate one in the Galyan River, and for indirect and non-contact recreational activities. In conclusion, the Değirmendere River currently supplying the potable water resource for Trabzon province is more polluted than the Galyan River.

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***In vitro* effects of metal ions on lipid peroxidation induced by alcohol in mice liver homogenate**

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Abstract : The study is aimed to estimate the effect of different heavy metals such as Hg^{2+} , Cd^{2+} , Mn^{2+} , Cr^{3+} , Ni^{2+} , $Se_2O_3^{2-}$, As_2O_3 water solution and combined effects of Hg^{2+} , Mn^{2+} , Cr^{3+} on lipid peroxidation in mice liver homogenate *in vitro*. Lipid peroxidation was determined as thiobarbituric acid-reacting materials (TBA). We select five different concentrations of selected ions for experiments. Correlations used to identify the concentration of ions associated with lipid peroxidation.

The rate of lipid peroxide formation in mice liver homogenate increased with the gradual addition of alcohol. When alcohol dose was up to 0.5 ml, the rate of lipid peroxide formation was greatest. At tested concentrations, the effects of metal ions on lipid peroxidation induced by alcohol were classified into three groups, and are as follows : (1) simulative, Hg^{2+} . (2) inhibitory, Mn^{2+} , Cr^{3+} , Ni^{2+} , $Se_2O_3^{2-}$. (3) ambiguous, Cd^{2+} , As_2O_3 water solution.

When Hg^{2+} , Mn^{2+} and Cr^{3+} were added to the mice liver homogenate with alcohol at the same time, Hg^{2+} , Mn^{2+} were the main agents for the rate of alcohol induced lipid peroxidation. The simulative effect of Hg^{2+} on lipid peroxidation induced by alcohol indicate that alcohol-drinkers will have further health risk when they are exposed in polluted regions than others, and Mn^{2+} , Cr^{3+} , Ni^{2+} , $Se_2O_3^{2-}$ may act as free radical scavengers and preventive remedy for alcoholism in part. Furthermore, analysis of combined effect of $Hg^{2+}Mn^{2+}$ and Cr^{3+} provide us a new way to estimate the combined effect of multi-materials.

Key words: Alcohol, Lipid peroxidation, Mice liver homogenate, Metal ions, Thiobarbituric acid method, *In vitro*.

Introduction

It is known that consumption of alcohol is a custom in many countries including China. It is reported that the production of alcohol and alcohol beverage was high to eight hundred million tons in 2001. Alcohol abuse has brought a lot of social and public hygiene problems, besides traffic incidents, force occurrence and heavy economic burden to either nation or individual, more serious it will bring many health disorders (Reynaud *et al.*, 2000; Harmeet *et al.*, 2000), even affects on human viability. Liver injury is the important damage caused by excessive drinking.

Alcohol can easily produce free radicals (Fang and Wenjie, 1989; Chen, 1993). Use of techniques such as spin trapping and EPR spectroscopy have demonstrably confirmed that both acute and chronic alcohol use by laboratory animals would generate free radical intermediates (Mufti *et*

al. 1993). Lipid peroxidation (LPO) is a free radical mediated process. Other researchers found that both acute and chronic alcohol intoxication are associated with an increase in LPO (Nordmann, 1994). So the rate of lipid peroxides can be taken as an indirect index to evaluate the free radical injury to organism originated by alcohol.

In a contemporary society, people have to absorb various metal ions unconsciously from eating, breathing, drinking and other ways everyday. The drinking people may suffer from the collective effect of alcohol and metal ions and other matter from environment, however, the study on relationships between metal ions and alcohol is not clear now. Some metal ions have been reported to show either simulative or inhibitory action on lipid peroxidation in microsomes or whole mice liver homogenates *in vitro*, there were different effects of metal ions in different systems inducing lipid peroxidation such as

the pollution parameters of the Değirmendere River were higher than those of the Galyan River and very close the limits. The results indicate that both Rivers can be used for the production of potable water during all seasons but only with an advanced treatment in the Değirmendere River and a moderate one in the Galyan River, and for indirect and non-contact recreational activities. In conclusion, the Değirmendere River currently supplying the potable water resource for Trabzon province is more polluted than the Galyan River.

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ascorbate, NADPH or CCl_4 (Yonaha *et al.*, 1980; Tam and McCay, 1970; Li and Shaofan, 2000). However, the roles of metal ions on lipid peroxidation induced by alcohol have not been well reported.

In view of the studies mentioned above, the present paper reports the respective and combined effects of metal ions on lipid peroxidation induced by alcohol in mice liver homogenate *in vitro*.

Materials and Methods

Materials : Male KunMing albino small mice (purchased from Experimental Animal Center, Institute of Inheritance, Chinese Academy of Sciences, Beijing, China) weighing about 30g were used. They were fed a normal diet and purified water. After 12 hours starvation, the animal was euthanised by decapitation after anesthetization, liver was taken out quickly, then washed blood on the surface of the liver by 0.9% NaCl solution, then blotted up the moisture by a filter paper. A sample of 2% (w/v) homogenates in 150 mmol/l cold KCl solution was obtained from the liver by an iced YQ-3 type apparatus.

Hg^{2+} , Cd^{2+} , Mn^{2+} , Cr^{3+} , Ni^{2+} , $\text{Se}_2\text{O}_3^{2-}$ and As_2O_3 were supplied in the formation of HgCl_2 , CdCl_2 , MnSO_4 , $\text{Cr}_2(\text{SO}_4)_3$, NiCl_2 and Na_2SeO_3 (Peking Chemical Co.), and As_2O_3 (Sigma Chemical Co.) water solution respectively. Alcohol in the study was purchased from Peking Chemical Co.; all chemicals and reagents employed were of commercial reagent grade quality.

Experimental procedure : Lipid peroxides were measured by the thiobarbituric acid (TBA) trichloroacetic acid method of Yonaha *et al.* (1980).

A sample of 1 ml 2% liver homogenate was taken into a 10 ml test tube by adding different dosage alcohol, purified water and ions solution with various concentrations, keeping the final volume at 3 ml. This mixture was incubated at 37°C for 30 min in a water bath. The reaction was stopped by transferring the test tubes to an ice bath followed by addition of 1 ml 20% trichloroacetic acid (TCA). Ten minutes later, the test tubes were centrifuged in a LXJ centrifuge (Product of Medicine Analytical Factory in Shanghai, China) for 10 min (3000

r/min), the 2.5 ml supernatant was drawn up to a test tube, and added 1.5 ml 0.67% TBA. The solution was then heated at 95°C for 10 min, then put in an ice bath for 5 min, then centrifuged for 5 min (2000 r/min) in the LXJ centrifuge to clarify the solution. Absorbency was measured at 532 nm, MDA concentration was represented by TBA optical density.

Data analysis : All test data were analyzed with the aid of Statistics 5.0 software. Data from the alcohol groups were compared with those of the control group using Student's 't' test. Single correlation analysis was performed between quantitative variables (Pearson's r test). Difference between the experimental groups was analyzed by using variance analysis by General Linear Model (GLM) - univariate procedure.

Results

Effects of various dosage of alcohol on lipid peroxidation : Table 1 shows that the rate of lipid peroxide formation in mice liver homogenate increases with the gradual addition of alcohol. When alcohol dosage was up to 0.5 ml, the rate of lipid peroxide formation got the peak, after that, with the increasing of alcohol dosage, the rate of lipid peroxide formation reduced. Namely, 0.5 ml alcohol has the highest ability inducing free radical reactions. As such, 0.5 ml alcohol was selected as the appropriate dosage for the following tests.

Effects of various metals on lipid peroxidation induced by alcohol : Figs. 1 to 7 illustrate the effects of Hg^{2+} , Cd^{2+} , Mn^{2+} , Cr^{3+} , Ni^{2+} , $\text{Se}_2\text{O}_3^{2-}$ and As_2O_3 water solution on lipid peroxidation induced by alcohol in mice liver homogenate. In the tested concentrations, TBA optical density showed a positive correlation with concentration of Hg^{2+} ($r = 0.995$, $p < 0.001$); TBA optical density of the group adding 0.5 mg/L Mn^{2+} has significant difference with that of alcohol group, hereafter, with increasing of Mn^{2+} concentrations, TBA optical density underwent no noticeable changes. Cr^{3+} , Ni^{2+} were inversely correlated with TBA optical density respectively ($r = -0.890$, $p < 0.05$; $r = -0.974$, $p < 0.01$). An inverse correlation was also observed changes with varieties of Cd^{2+} , As_2O_3 water solution concentrations. In

In vitro effects of metal ions on lipid peroxidation.

Table - 1 : Effects of various dosage of alcohol on lipid peroxidation.

Alcohol dosage (ml)	Peroxides formed (TBA optical density)	T test with control group
0 (control group)	0.185 ± 0.006 (3)	—
0.1	0.178 ± 0.008 (3)	- 1.2124
0.3	0.238 ± 0.008 (3)	9.1799*
0.5	0.284 ± 0.011 (3)	13.685**
0.7	0.130 ± 0.003 (3)	- 14.2009**
1.0	0.081 ± 0.003 (3)	-26.8527**

The data are given as mean ± standard deviation (repeating time).

Data from the alcohol groups were compared with those of the control group using student's *t* test.

* $p < 0.05$, ** $p < 0.01$

Table - 2 : Combined effects of Hg^{2+} , Mn^{2+} and Cr^{3+} on lipid peroxidation induced by alcohol.

No	Group	Peroxides formed (TBA optical density)
1	Control	0.324 ± 0.009 (4)
2	Alcohol (0.5ml)	0.460 ± 0.008 (4) ^a
3	Alcohol (0.5ml) + Hg^{2+} (10 mg/L)	0.669 ± 0.007 (4) ^a
4	Alcohol (0.5ml) + Mn^{2+} (10 mg/L)	0.128 ± 0.002 (4) ^b
5	Alcohol (0.5ml) + Cr^{3+} (10 mg/L)	0.253 ± 0.002 (4) ^b
6	Alcohol (0.5ml) + Hg^{2+} (10 mg/L) + Mn^{2+} (10 mg/L)	0.154 ± 0.015 (4) ^b
7	Alcohol (0.5ml) + Hg^{2+} (10 mg/L) + Cr^{3+} (10 mg/L)	0.588 ± 0.016 (4) ^b
8	Alcohol (0.5ml) + Mn^{2+} (10 mg/L) + Cr^{3+} (10 mg/L)	0.126 ± 0.003 (4) ^b
9	Alcohol (0.5ml) + Hg^{2+} (10 mg/L) + Mn^{2+} (10 mg/L) + Cr^{3+} (10 mg/L)	0.168 ± 0.012 (4) ^b

The data given as mean ± standard deviation (repeating time).

a : data from testing groups were compared with those of the group 1 using student's *t* test.

b : data from testing groups were compared with those of the group 2 using student's *t* test.

short, for the lipid peroxidation induced by alcohol, Hg^{2+} shows an enhancing effect, Mn^{2+} , Cr^{3+} , Ni^{2+} and $Se_2O_3^{2-}$ have inhibitory effects whereas Cd^{2+} and As_2O_3 water solution effects were ambiguous.

Combined effect of Hg^{2+} , Mn^{2+} and Cr^{3+} on lipid peroxidation induced by alcohol : From the seven ions used in this experiment, three ions that had significant effects (inhibitory or enhancing effect) on lipid peroxidation induced by alcohol were selected to study the combined effect of metal ions. The results are shown in Table 2. Alcohol can induce lipid peroxidation in mice liver homogenate *in vitro*. The addition of 10 mg/L Hg^{2+} (group 3) significantly increased in LPO 45.4% than that of alcohol group, moreover adding of 10 mg/L Mn^{2+} (group 6) and 10 mg/L Cr^{3+} (group 7) significantly reduced in LPO 76.9% and 12.1% than that of alcohol- Hg^{2+} group, respectively. Adding of 10 mg/L Mn^{2+} (group 4) and

10 mg/L Cr^{3+} (group 5) respectively decreased in LPO 72.2% and 45% than that of alcohol group. Adding of Mn^{2+} and Cr^{3+} at the same time (group 8) significantly decreased 72.6% in LPO than that of alcohol group (group 2). Adding of 10 mg/L Hg^{2+} , Mn^{2+} and Cr^{3+} simultaneously (group 9) reduced in LPO 63.5% than that of alcohol group (group 2). In order to elucidate the interaction of Hg^{2+} , Mn^{2+} and Cr^{3+} in free radical reactions induced by alcohol in mice liver homogenate *in vitro*, an Uni-variate Analysis of Variance analysis was completed for original results (group 2-9 in Table 2), where results are summarized in Table 3.

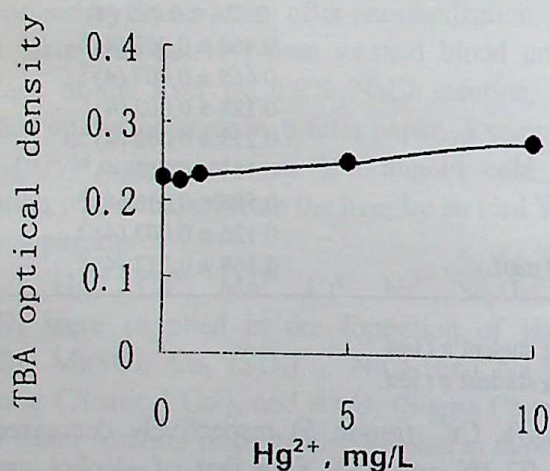
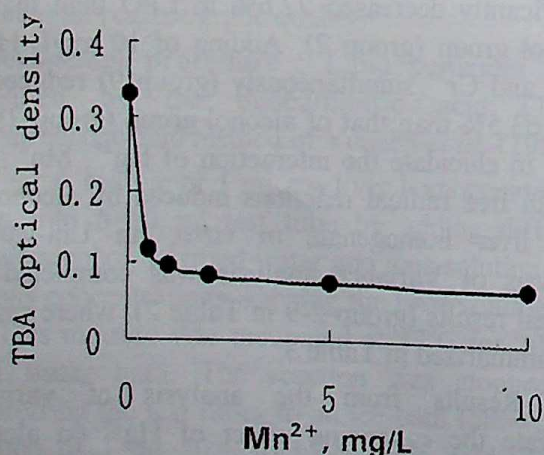
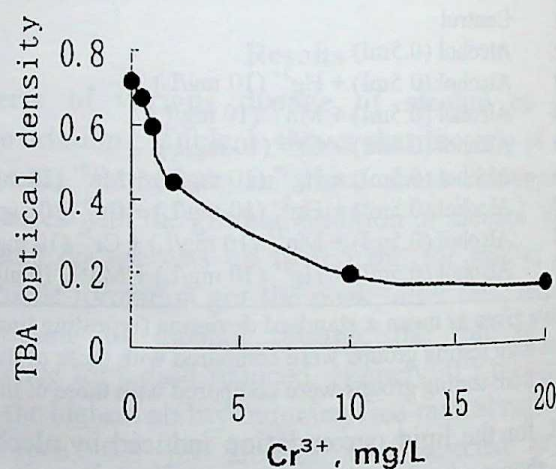
Results from the analysis of variance illustrate the enhancing effect of Hg^{2+} on alcohol induced lipid peroxidation [$F(1,7) = 721.5$, $p = 0.000$]. Conversely Mn^{2+} and Cr^{3+} have inhibitory effects [$F(1,7) = 6171.5$, $p = 0.000$; $F(1,7) = 84.8$;

Table - 3 : Uni-variate Analysis of Variance (effects and combined effects of Hg^{2+} , Mn^{2+} and Cr^{3+} on lipid peroxidation induced by alcohol).

Source	Sum of squares	df	Mean square	F	Sig.	(Eta) ²
Corrected Model	1.356 ^a	7	0.194	1069.464	0.000	0.997
Intercept	3.5	1	3.500	19322.54	0.000	0.999
Hg^{2+}	0.131	1	0.131	721.495	0.000	0.968
Mn^{2+}	1.118	1	1.118	6171.55	0.000	0.996
Cr^{3+}	1.54E-02	1	0.0154	84.778	0.000	0.779
$\text{Hg}^{2+} \text{ Mn}^{2+}$	7.10E-02	1	0.071	391.808	0.000	0.942
$\text{Hg}^{2+} \text{ Cr}^{3+}$	8.93E-04	1	0.000893	4.927	0.036	0.170
$\text{Mn}^{2+} \text{ Cr}^{3+}$	2.02E-02	1	0.0202	111.244	0.000	0.823
$\text{Hg}^{2+} \text{ Mn}^{2+} \text{ Cr}^{3+}$	8.13E-05	1	0.0000813	0.449	0.509	0.018
Error	4.35E-03	24	0.000181			
Total	4.86	32				
Corrected Total	1.36	31				

Dependent Variable : TBA optical density. Fixed variables : Hg^{2+} , Mn^{2+} , Cr^{2+}

a. R Squared = .997 (Adjusted R Squared = .996), Eta : estimates of effect size.

**Fig. 1 :** Relationship between TBA optical density and Hg^{2+} concentrations.**Fig. 2 :** Relationship between TBA optical density and Mn^{2+} concentrations.**Fig. 3 :** Relationship between TBA optical density and Cr^{3+} concentrations.

$p = 0.000$]. There exists combinative effect between Hg^{2+} and Mn^{2+} [$F(1,7) = 391.808$, $p = 0.000$], Hg^{2+} and Cr^{3+} [$F(1,7) = 4.927$, $p = 0.036$] and Mn^{2+} and Cr^{3+} [$F(1,7) = 111.244$, $p = 0.000$] but the collective effect of Hg^{2+} , Mn^{2+} Cr^{3+} [$F(1,7) = 0.449$, $p = 0.509$] is not significant. Certain ion or two combined ions may contribute to the effect. Compared each experimental group to the total variance (Eta), effect sizes are $\text{Mn}^{2+} > \text{Hg}^{2+}$, $\text{Mn}^{2+} > \text{Mn}^{2+}$, $\text{Cr}^{3+} > \text{Cr}^{3+}$ $> \text{Hg}^{2+}$, $\text{Cr}^{3+} > \text{Hg}^{2+}$, Mn^{2+} and Cr^{3+} , which indicated that when Hg^{2+} , Mn^{2+} and Cr^{3+} are added into the alcohol reaction system together, Hg^{2+} and Mn^{2+} are the main factors for lipid peroxidation induced by alcohol in mice liver

In vitro effects of metal ions on lipid peroxidation.

homogenate *in vitro*, that means, the interaction between the two ions stands for the interaction among the three ions.

Discussion

Many observations have shown that alcohol induced liver injury may be linked to oxidative stress resulting from increased free radical production and decreased antioxidant defense (Nordmann *et al.*, 1994). A comparison of the contents of V_C , V_E , and β -carrot in plasma, where activities of enzymes such as SOD, CAT, GSH in red cell in alcoholics and non-consumer of alcohol, indicate that alcohol consumption may conduct oxidant and antioxidant system imbalance in human body (Zhou and Yuhong, 1998). In the present study, appropriate addition of alcohol in mice liver homogenate will induce free radical reactions, however, the excessive alcohol (>0.5 ml) decreased the rate of lipid peroxidation. Such results were also observed in experiments of Yang and Xuemin (1996).

Mercury is an important environmental pollutant; mercuric chloride decreases the activity of glutathione peroxidase, which catalyzes the destruction of organic hydro peroxides (Yonaha *et al.*, 1980). Herein, we observed a marked positive correlation between concentrations of Hg^{2+} with the rate of lipid peroxidation in the presence of alcohol ($r = 0.995$, $p < 0.001$). This stimulatory effect of Hg^{2+} demonstrated that Hg^{2+} aggravated lipid peroxidation induced by alcohol.

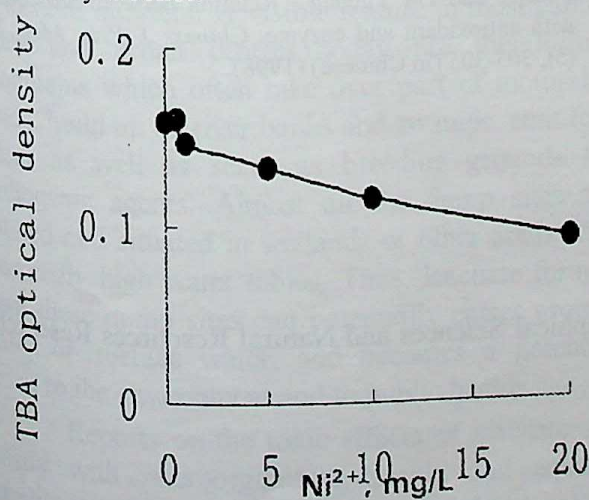


Fig. 4 : Relationship between TBA optical density and Ni^{2+} concentrations.

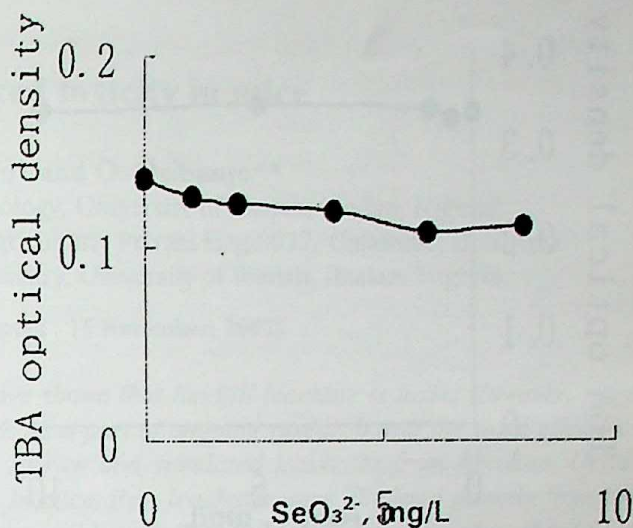


Fig. 5 : Relationship between TBA optical density and $Se_2O_3^{2-}$ concentrations.

In the present alcohol induced lipid peroxidation system, the actions of Cd^{2+} and As_2O_3 were ambiguous.

Mn and Se are necessary life elements, which have important actions on related enzymes such as SOD and GSH- P_x . Mn, Ni and Cr can exist in multiple oxidation states, which easily gain and lose electrons (Dean, 1995; Zhang *et al.*, 1985), and are probably taken as inhibitor of free radical reaction (Tam and McCay, 1970). The potent inhibitions of Mn, Se, Cr and Ni on lipid peroxidation in the presence of alcohol in liver homogenate were observed. The results suggest that Mn^{2+} , $Se_2O_3^{2-}$, Cr^{3+} and Ni^{2+} may act as free radical

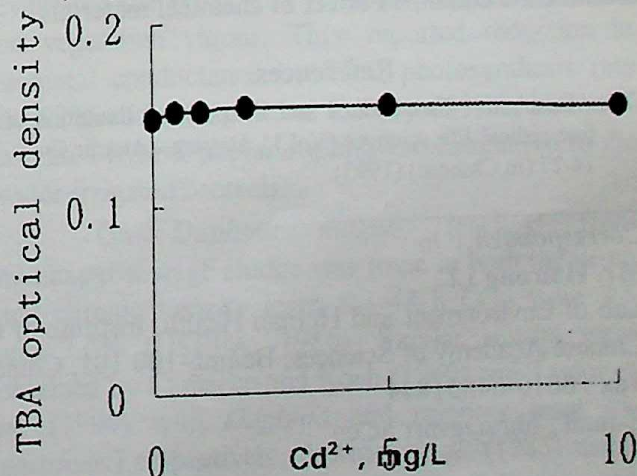


Fig. 6 : Relationship between TBA optical density and Cd^{2+} concentrations.

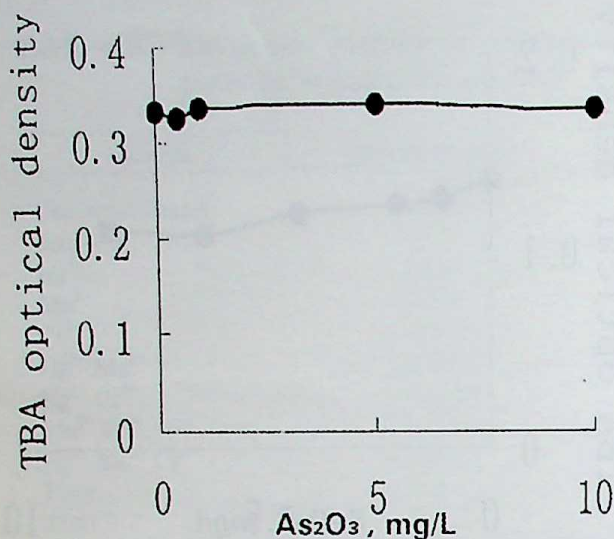


Fig. 7 : Relationship between TBA optical density and As₂O₃ water solution concentrations.

scavengers and preventive remedy for alcoholism in part, however, it should be validated *in vivo* experiment, and the dosage and formation of the ions should be carefully selected.

Through analysis of the combined effect of Hg²⁺, Mn²⁺ and Cr³⁺ on lipid peroxidation induced by alcohol, we found that these three ions associated effect was not equal with summation of independent effect of every ion, but processed a ions' reciprocation. The coexisting effect of these three ions can be simplified into the combined effect of two ions, which greatly simplifies the research in a mechanism of coexisting effects of metal ions. More work should be done to provide a new method to estimate the combined effect of chemical materials.

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Landfill leachate-induced toxicity in mice

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Abstract : Microbial, plant and studies in aquatic animals have shown that landfill leachate is toxic. However, more information about its effects in terrestrial animals is required. As a part of ongoing research into the toxic effects of landfill leachate in Nigeria, we evaluated the acute effects of raw and simulated leachates from Abadina, Orita-Aperin and Oworonsoki dump sites, all in Southwest Nigeria, in mice. Raw leachates were obtained directly from the dumps while the simulated leachates were obtained from the solid wastes in the laboratory by using the ASTM method. The samples were designated Abadina raw leachate (ARL), Orita-Aperin raw leachate (OARL) and Oworonsoki raw leachate (OWRL); and Abadina simulated leachate (ASL), Orita-Aperin simulated leachate (OASL) and Oworonsoki simulated leachate (OWSL). Their physico-chemical properties were determined in accordance with standard analytical methods. Young male mice (12-15 wk) weighing 24-31g were exposed to 1%, 5%, 10%, 25%, 50% and 100% concentrations of each test samples for 5 consecutive days and were observed for a period of 96 h for toxic response. Mortality recorded at different times for each sample at the various concentrations was mostly within the last 48 h of the exposure period. The LC_{50} obtained are 100% for both ARL and OARL, and 50% for OWRL; and 83.50% and 50% for ASL and OWSL, respectively. It was indeterminate for OASL. Apart from this, other toxic effects like weight loss, sluggishness, loss of hair and reduced food intake were observed. The investigated samples were ranked as OWRL > OWSL > ASL > OARL > ARL > OASL. The observed effects were due to the toxic constituents present in the leachate samples. This suggests that the mixtures have the potential to cause harmful effect to public health and our environment through seepage into ground or surface water.

Key words : Raw and simulated leachate, Dump sites, Acute toxicity, Albino mice.

Introduction

Most solid waste dump sites in Nigeria are located in public places and are surrounded by residential quarters. A visible feature in most capital cities and urban centres today are the Refuse Mountains which often take over part of motorable roads; build up on river banks and swamps, emit foul odors as well as serve as breeding grounds for pathogenic agents. Almost all the dump sites are unlined and situated in wetlands or other areas with seasonally high water tables. Thus, leachate formed from these dump sites can potentially enters ground water or surface water, and becomes a potential threat to the environment and to public health.

Reports on the toxic effects of leachate are mostly with microorganisms, plants and aquatic animals. Cheung *et al.* (1993) reported that leachate from two landfills inhibited the growth rates ($P < 0.05$) of four species of microalgae. Similar result

was obtained with radish, sorghum, wheat and soybean roots and shoot growth after treatment with waste extract (USEPA, 1980). Cureton *et al.* (1991) evaluated the effect of two MSW landfill leachates on vegetation vigour. They reported reduction in stomatal conductance by 73%, photosynthesis rate by 63% and transpiration rate by 68% in leachate-irrigated hybrid poplar sapling leaves relative to the water-irrigated controls.

On *Daphnia magna*, leachate from municipal sewage sludge was toxic in both the acute and chronic toxicity tests, the 48 h LC_{50} were 7.5 and 3.0% (USEPA, 1980). Similar results were obtained by Cameron and Koch (1980) and Taylor *et al.* (1996) with *Daphnia* and rainbow trout (*S. gairdneri*) respectively, Atwater *et al.* (1983) using *D. pulex*, *S. gairdneri* and *O. nerka* as test organisms, and Bernard *et al.* (1996) using a battery of conventional toxicity tests (microalgae,

duckweeds, daphnids) and new microbiotests (rotifers, crustaceans, protozoan, luminescent bacteria). Contrarily, Rutherford *et al.* (2000) reported that the final leachate from a municipal landfill using a wetland tertiary treatment system were not acutely toxic to rainbow trout (*O. mykiss*), *D. magna* and *V. fischeri*. Benthic macroinvertebrate sampling however, revealed that the leachate discharge had a localized impact on community structure.

We studied the acute and mutagenic effects of raw and simulated leachates from three dump sites in southwestern Nigeria in *A. cepa* (Bakare *et al.*, 1999; 2000a; 2000b). Our results showed significant inhibition of root growth ($P < 0.05$) and induction of different types of chromatid and chromosome type of structural aberration at different concentrations of the tested samples. This was attributed to the presence of toxic chemicals in the tested samples. In this report, we evaluated the toxic effects of the raw and simulated leachate samples from Abadina, Orita-Aperin and Oworonsoki dump sites in Southwest Nigeria in albino mice.

Materials and Methods

Sampling site, leachate collection, and simulation from solid waste : Three dump sites, Abadina, Orita-Aperin and Oworonsoki, in Oyo and Lagos states, South western Nigeria were used in this study. The Abadina dump sites, at the University of Ibadan, Nigeria contains wastes from residential quarters, laboratories and offices in the University. The Orita-Aperin dump site was an old, spent MSW dump site, located in the S4, S5 wards (Ayeni, 1994) of Ibadan North East Local government area of Ibadan (Longitude 3°5', latitude 7°23'), Nigeria. Likewise, Oworonsoki dumpsite in Somolu Local government area of Lagos State (Longitude 3°24', latitude 6°27') receives wastes from domestic (30%) and industrial (70%) sources.

Raw leachate was collected from leachate wells at Abadina and Oworonsoki dumps. At Orita-Aperin, samples were collected from 10 different spots where leachate seeps out of the dump. They were designated Abadina Raw Leachate (ARL), Orita-Aperin Raw Leachate (OARL) and

Oworonsoki Raw Leachate (OWRL). For leachate simulation, solid wastes collected from these waste dumps in the dry seasons of 1996 and 1997, were shredded to provide representative sample for simulation using the ASTM method, which is a category A extraction procedure (Perket *et al.*, 1982). The procedure, with slight modification was described in our study with *A. cepa* (Bakare *et al.*, 2000a). The samples designated as Abadina Simulated Leachate (ASL), Orita-Aperin Simulated Leachate (OASL), Oworonsoki Simulated Leachate (OWSL) and the raw samples were filtered to remove debris, pH was taken and stored at 4°C.

The physico-chemical properties of the leachate samples and tap water were determined in accordance with standard analytical methods (APHA, 1985). The metals were analysed with atomic absorption spectrophotometer.

Acute toxicity assay : Male mice weighing 24-31 g obtained from Institute for Advanced Medical Research and Training, University College Hospital, Ibadan, Nigeria were used for this study. Six concentrations of 1%, 5%, 10%, 25%, 50% and 100% of each leachate sample were tested using 8 mice in each group. Each mouse in each group was given a single intraperitoneal injection of 0.5 ml of the test samples for 5 consecutive days. The negative and positive control received the same volume, but normal saline and tap water respectively, as the treated mice. They were observed for a period of 96 h for their response to the injected leachate. Death was used as a criterion of a change in this test. Other toxic responses shown by the animals were also recorded. From the values obtained, the LC_{50} for each leachate sample was obtained by the logarithmic method.

Results and Discussion

Table 1 summarizes the percentage survivors and mortality of the animals in the control and the test groups. From this table the 96 h LC_{50} of each sample was obtained as 100% for both ARL and OARL, 50% for both OWRL and OWSL, and 83.5% for ASL. It was indeterminate for OASL (Figs. 1 and 2).

Landfill leachate-induced toxicity in mice.

With ARL, the LC_{50} of 100% showed that the test animals were able to tolerate the various concentrations of this sample until the 100% concentration where 50% mortality was recorded within the last 24 h of the exposure period. However, ASL LC_{50} of 83.5% shows it to be more toxic than ARL in this test. It caused 16.67% and 66.70% mortality at the 50% and 100% concentrations, respectively. With Orita-Aperin leachate, the raw sample was more toxic. It caused no death and no severe physiological disorders at 1% and 5% concentrations, but it induced 16.67%, 16.67%, 33.30% and 50% mortality at the 10%, 25%, 50% and 100% concentrations respectively. Other symptoms such as loss of hair and slow movement of the test animals were observed at the 50% and 100% concentrations of OARL; and at the 100% concentrations of OASL where there was 33.30% mortality. Both OWRL and OWSL have similar LC_{50} 's of 50% and similar toxic effects such as reduced food intake and loss of hair in the test groups. The severity of the effects was seen at the 100% concentration where only two mice survived

for both samples. The raw leachate was however, more toxic based on the concentration at which the test animals started showing signs of toxic effects.

Altogether, no lethal effects were seen at the 1% and 5% concentrations of the tested samples. For the raw samples, ARL did not induce any lethal effect until the highest concentration where there was 50% mortality. OARL and OWRL caused death of mice at the same concentrations of 10%, 25%, 50% and 100% but OWRL was more toxic based on the number of death recorded at the end of the exposure period. Its LC_{50} makes it the most toxic of the raw leachate samples (Fig. 1). For ASL and OWSL no mortality was recorded until the 50% concentration and at the 25% concentration for OASL. While mice at the higher concentrations of ASL did not show other toxic signs apart from death, mice at the higher concentrations of OASL exhibited some physiological disorders such as loss of hair and weight. Of the three simulated samples, OWSL was the most toxic with its low LC_{50} of 50% and other toxic effects induced in tested animals.

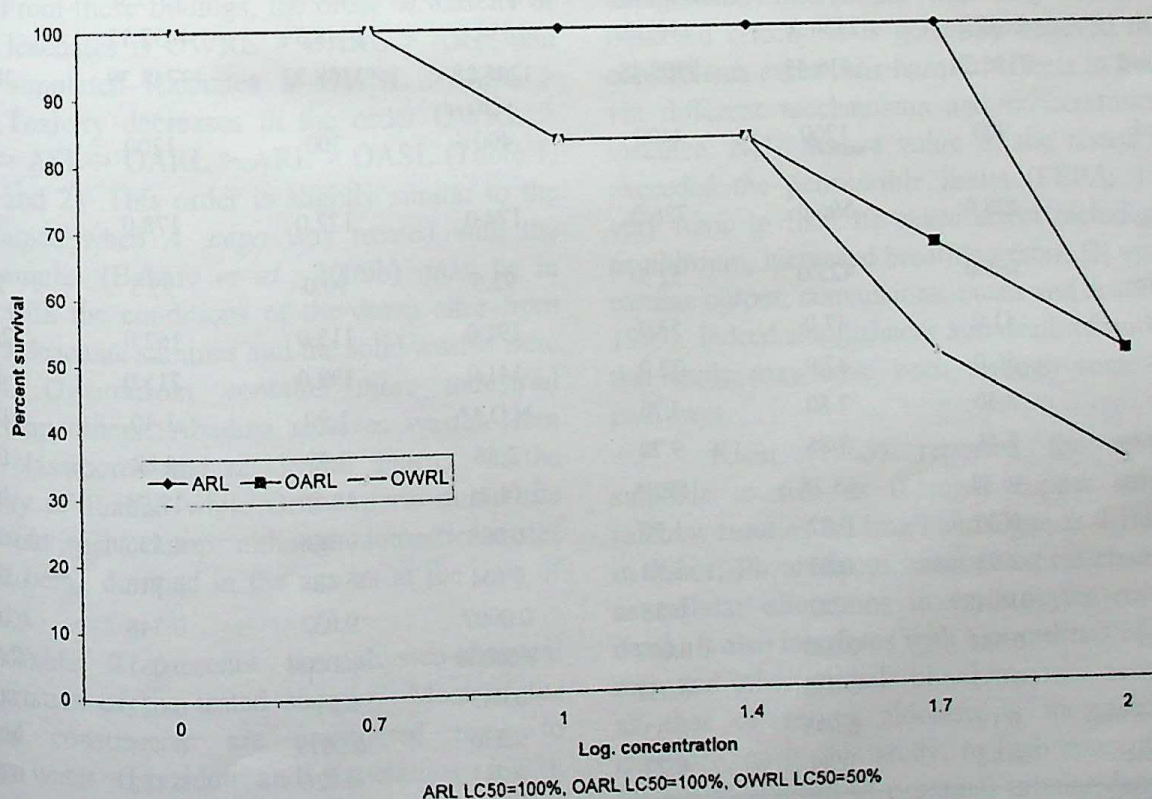


Fig. 1 : The effect of Abadina, Orita-Aperin and Oworonsoki raw leachate on mice.

Table – 1 : Summary of toxic effects of Abadina, Orita-Aperin and Oworonsoki raw and simulated leachates on mice.

Conc. (%)	Raw leachates						Simulated leachates					
	ARL		OARL		OWRL		ASL		OASL		OWSL	
	%	%	%	%	%	%	%	%	%	%	%	%
	Mortality	Survivors	Mortality	Survivors	Mortality	Survivors	Mortality	Survivors	Mortality	Survivors	Mortality	Survivors
Control	0	100	0	100	0	100	0	100	0	100	0	100
1	0	100	0	100	0	100	0	100	0	100	0	100
5	0	100	0	100	0	100	0	100	0	100	0	100
10	0	100	16.7	83.30	16.7	83.30	0	100	0	100	0	100
25	0	100	16.7	83.30	16.7	83.30	0	100	16.7	83.30	0	100
50	0	100	33.3	66.67	50	50	16.7	83.30	16.7	83.30	50	50
100	50	50	50	50	66.7	33.30	66.7	33.30	33.3	66.67	66.7	33.30
LC ₅₀	100%		100%		50%		83.50%		Indeterminate		50%	

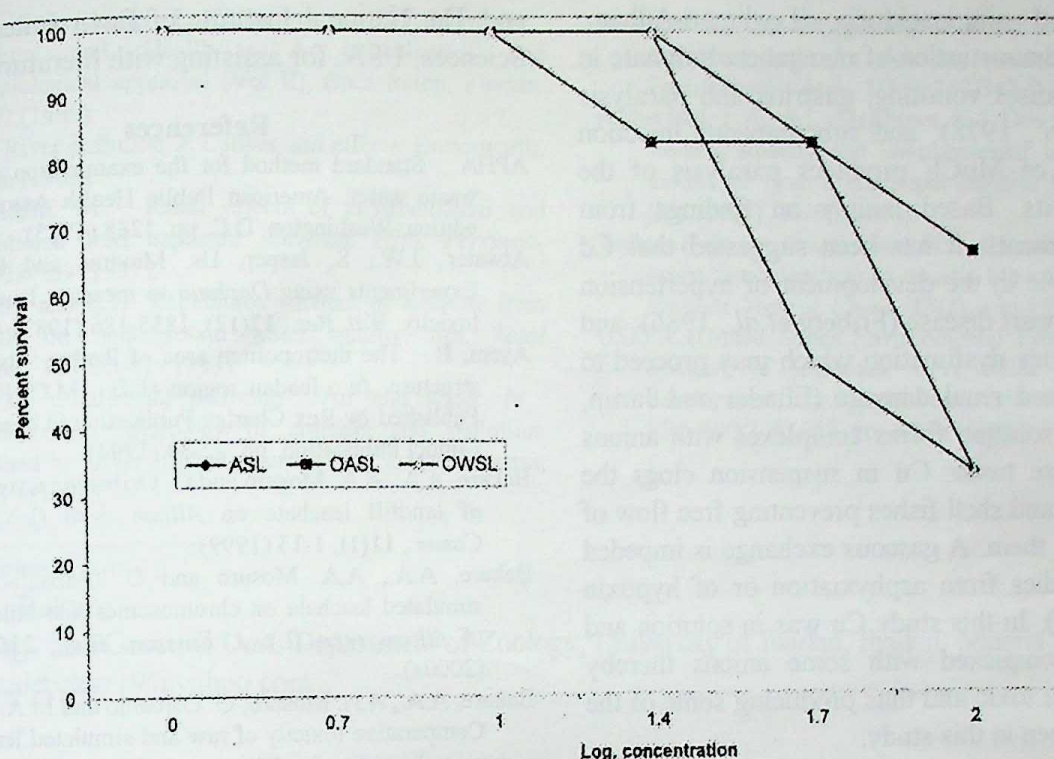
Table – 2 : Physical and chemical characteristics of the leachate samples and tap water.

Parameters*	ARL	OARL	OWRL	ASL	OASL	OWSL	Tap water
pH	7.3	7.7	7.8	6.6	6.9	7.2	7.8
Colour	Pale yellow	Dark brown	Dark brown	Pale brown	Brown	Brown	Colorless
BOD	755.1	716.0	904.8	843.0	1045.0	1352.0	-
COD	2647.2	2534.5	2891.5	2634.0	2448.39	4037.12	-
Total solid	3186.24	5436.43	9969.48	1246.28	1138.22	3748.39	288.63
Total dissolved solid	900	1700	4400	400	300	1200	100
Total Hardness	428.0	596.0	726.0	174.0	172.0	178.0	3.0
Total Alkalinity	165.0	425.0	52.5	92.5	90.0	97.5	22.5
Chloride	41.0	47.0	53.0	191.0	115.0	162.0	232.0
Sulphate	56.0	87.0	72.0	141.0	198.0	215.0	41.0
Nitrate	3.30	7.80	4.75	N.D.**	1.90	1.40	0.08
Ammonium	6.41	5.86	9.70	2.56	3.97	4.82	0.18
Magnesium	46.41	68.28	59.05	18.71	18.50	17.74	0.49
Copper	0.06	0.12	1.30	0.068	0.068	0.136	0.000091
Iron	1.95	0.62	6.94	6.651	6.488	6.716	0.00014
Lead	0.0048	0.22	0.38	0.0007	0.032	0.348	0.00037
Cadmium	0.024	0.036	0.077	0.00008	0.00004	0.061	0.000018
Silver	0.032	0.032	0.032	0.043	0.011	0.022	0.000047
Nickel	0.19	0.095	0.33	0.236	0.0019	0.377	0.188
Manganese	0.48	0.34	0.27	0.711	0.726	0.193	0.0689

* All values are in mg/l except pH

** ND = Not detectable

Landfill leachate-induced toxicity in mice.



ASL LC50=83.50, OASL LC50=Indeterminate, OWSL LC50= 50%

Fig. 2 : The effect of Abadina, Orita-Aperin and Oworonsoki simulated leachate on mice.

From these findings, the order of toxicity of the raw leachates is OWRL > OARL > ARL; and for the simulated leachates is OWSL > ASL > OASL. Toxicity decreases in the order OWRL > OWSL > ASL > OARL > ARL > OASL (Table 1, Figs. 1 and 2). This order is slightly similar to the one obtained when *A. cepa* was treated with the tested samples (Bakare *et al.*, 2000b), may be in concert with the conditions of the dump sites from which the leachate samples and the solid wastes were obtained. Oworonsoki contains more industrial wastes than others; Abadina receives wastes from offices, classrooms and residential quarters in the University of Ibadan while Orita-Aperin dump site was an old spent dump, although domestic wastes was still being dumped in the site as at the time of this study.

Table 2 presents the physico-chemical characteristics of the tested samples. Most of the identified constituents are considered toxic to drinking water (Loizidou and Kapetanios, 1993). Their presence and those of other unidentified constituents and their individual, synergistic and or

antagonistic interaction was responsible for the observed effects. It is generally believed that these constituents exert their harmful effects in living cells via different mechanisms and concentrations. For instance, NH_3 , whose value in the tested samples exceeded the permissible limits (FEPA, 1991), is very toxic to fish. Its acute effect includes loss of equilibrium, increased breathing rates, O_2 uptake and cardiac output, convulsions, coma and death (Odiete, 1999). Indeed sluggishness and death of mice seen in this study may have been through some of these pathways.

Klein (1965) reported the toxicity of sulphide to fish as 0.5mg/l, copper sulphate to rainbow trout as 0.14mg/l and algae as 0.1mg/l. Also in fishes, Pb produces haematological changes such as cellular alterations in erythrocytes resulting to death. It also interferes with biosynthesis of haem. In man and other animals blood enzymes activity such as that of serum aldolase is increased. Acute exposure, as in this study, to high concentration of Pb can also result in proximal tubular damage with characteristic histologic features and manifested by

glycosuria and aminoaciduria (Loghman-Adham, 1997). Oral administration of manganese sulphate in acute doses causes vomiting, gastritis and paralysis (Fishbein *et al.*, 1978); and subcutaneous injection of 10-16 mg of $MnCl_2$ produces paralysis of the CNS in rabbits. Based mainly on findings from animal experiments, it has been suggested that Cd may play a role in the development of hypertension and ischemic heart disease (Friberg *et al.*, 1986); and in tubular kidney dysfunction which may proceed to more generalized renal damage (Elinder and Jarup, 1996). Cu in solution forms complexes with anions making it more toxic. Cu in suspension clogs the gills of fishes and shell fishes preventing free flow of water through them. A gaseous exchange is impeded and the fish dies from asphyxiation or of hypoxia (Odiese, 1999). In this study Cu was in solution and could have complexed with some anions thereby making it more toxic and thus producing some of the toxic effects seen in this study.

It is believed that the exposed mice accumulated these toxicants in their tissues and organs most especially the kidney, heart and liver. This subsequently led to death and other toxic symptoms via any or combination of the above mechanisms. Our study is similar to those of Dura and Horvath (1988) wherein mice were exposed intraperitoneally to aqueous leachates as well as extracts of wastes of metal, machinery, leather and chemical industry, and municipal sludge in Hungary.

We are not aware of any other report from Africa on landfill leachate toxicity in terrestrial animals. Thus, this study may serve as a guide for the toxicological assessment of leachate samples in terrestrial animals. At present, there is no method of study satisfying every requirement for the toxicological studies of the combined effect of chemicals (Dura and Horvath, 1988) in living systems. Further studies on the mutagenicity and effect of the leachate samples on reproductive cells in mice are in progress.

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The effects of compost prepared from waste material of banana plants on the nutrient contents of banana leaves

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Abstract : In this study, the possible utilization of removed shoots and plant parts of banana as compost after fruit harvest were investigated. Three doses (15-30-45 kg plant⁻¹) of the compost prepared from the clone of Dwarf Cavendish banana were compared with Farmyard manure (50 kg plant⁻¹), Mineral fertilizers (180 g N+150 g P+335 g K plant⁻¹) and Farmyard manure + Mineral fertilizers (25 kg FM+180 g N+150 g P+335 g K plant⁻¹) which determined positive effects on the nutrient contents of banana leaves. The banana plants were grown under a heated glasshouse and in a soil with physical and chemical properties suitable for banana growing. The contents of N, P, K and Mg in compost and in farmyard manure were found to be similar. Nitrogen, phosphorus and potassium contents of leaves in all applications except control, and Ca, Mg, Fe, Zn, Mn, Cu contents in all applications were determined between optimum levels of reference values. There were positive correlations among some nutrient contents of leaves, growth, yield and fruit quality characteristics.

Farmyard manure, Farmyard manure + Mineral fertilizers and 45 kg plant⁻¹ of compost increased the nutrient contents of banana leaves. According to obtained results, 45 kg plant⁻¹ of compost was determined more suitable in terms of economical production and organic farming than the other fertiliser types.

Key words : Banana, Compost, Fertilization, Plant nutrient, Organic farming.

Introduction

Banana originates from Southeast Asia and is a tropical climate fruit. It has been one of the most important foods of people for hundreds of years due to containing some very specific carbohydrates, vitamins and mineral nutrients (Onur, 1990).

In Turkey, banana is produced generally as Dwarf Cavendish cultivar in Alanya, Gazipaşa, Anamur, Bozyazı and Erdemli districts (Onur, 1990). In 1997, banana growing was realized on 1165 ha, and total production was 32600 tons (A. S. P. 1997).

According to Lahav and Turner (1983), from one hectare, banana plants took up 450 kg N, 60 kg P₂O₅, 1500 kg K₂O, 215 kg CaO, 140 kg MgO, 12 kg Mn, 5 kg Fe, 1.5 kg Zn, 1.25 kg B and 0.5 kg Cu for 50 tones yield in a year. De Geus (1967) suggested that Dwarf Cavendish, grown under Papua New Guinea conditions took up 1.73 kg N, 0.46 kg P, 6.0 kg K, 0.17 kg Ca and 0.32 kg Mg by one-ton yield.

In banana production, higher amount of potassium and nitrogen among macro nutrients (K : N ratio) is most important for optimum yield. After N and K, Mg is the third most important nutrient for banana plants, followed by P, Zn and Mn. Therefore, for high yield with better quality in banana production, there should be a balance and adequate nutrients (Özbek and Danişman, 1978; Martin-Prevel *et al.*, 1987). In banana plantings, after fruit harvest, removed shoots and old plant parts are thrown out in every spring. This waste material can be a secondary host of diseases and pests, and also requires additional expenditure to transport it further away (Onur, 1990).

This study was carried out under glasshouse conditions in the Erdemli County of the İçel Province. The possible utilisation of removed shoots and plant parts of banana after fruit harvest as compost were investigated. The obtained results were compared with those obtained for organic and inorganic fertilizers.

Materials and Methods

This study was carried out during the years of 1993-1996 under a heated glasshouse of the Horticulture Research Institute in İçel, Turkey, with a Dwarf Cavendish clone. The experiment was performed as randomised blocks design with three replications and with two banana plants in each parcel. Parcel size was 12.25 m². The data obtained were statistically analysed by using the Tarist (Ege University, Izmir, Turkey) software. The means of analysis findings were compared by LSD test. Relationships among growth, yield, quality characteristics and nutrients of leaves were examined by correlation analysis (Açıkgöz, 1993).

In experiments, three doses of compost (15-30-45 kg plant⁻¹), farmyard manure (50 kg plant⁻¹), mineral fertilizers (180 g N + 150 g P + 335 g K plant⁻¹) and farmyard manure + mineral fertilizers (25 kg FM+180 g N+150 g P+335 g K plant⁻¹) were applied in 1995 and 1996. The drip irrigation method was used, and all of compost doses, farmyard manure and triple super phosphate fertilizers were applied in March, whereas, ammonium sulphate was applied in three stages : 1/3 in March, 1/3 in June, 1/3 in July. Potassium sulphate was applied in two stages : 1/2 in March, 1/2 in June (Özbek, 1981).

In November 1993, soil samples were taken from 0-30 cm and 30-60 cm depth and were analysed according to the following methods. Soil texture was determined by using hydrometer method (Bouyoucos, 1955) ; pH with glass pH meter on saturated soil (Jackson, 1958) ; total soluble salt with electrical conductivimeter on saturated soil (S. L. S., 1954) ; lime with Scheibler calcimeter (Martin and Reeve, 1955) ; organic matter by Walkley-Black method (Jackson, 1958) ; total N by semi micro-Kjeldahl

method (Chapman *et al.*, 1961) ; available P by sodium bicarbonate blue-colour method (Olsen and Deal, 1965) ; exchangeable cations (K, Ca, Mg) by ammonium acetate method (Jackson, 1958) ; available Zn, Fe, Mn, Cu by DTPA method (Lindsay Norwell, 1978).

Before starting the study Dwarf Cavendish banana plants were grown in the experimental glasshouse, so the leaf samples from the banana plants, which were used to prepare compost, were taken to determine general nutrition situation in November 1993. Leaf samples were taken from both sides of the lamina and at its exact mid-length, from the third youngest fully expanded leaf.

The samples were obtained by cutting a 10 cm long piece of midrib exactly half way along the leaf (Lahav and Turner, 1983) and were analysed according to the following methods. Total leaf N contents were determined by the Kjeldahl method (Chapman *et al.*, 1961) and P by vanadate-molybdate yellow colorimetric method using spectrophotometer (Barton, 1948). The leaf contents of K, Ca, Mg, Fe, Zn, Mn and, Cu were assessed by atomic absorption spectrophotometer (Perkin Elmer-372) after ashing at 550°C and extraction in 10% hydrochloric acid (Chapman *et al.*, 1961).

The analysis results of leaf sample of banana plants are given in Table 1.

As seen in Table 1, the nutrient contents in the leaf sample of banana plants used to prepare compost were determined between optimum levels of reference values given by Lahav and Turner (1983).

Compost was prepared by cutting the banana plants' removed shoots and plant parts put out after fruit harvest (in March 1994) into the small

Table - 1 : The nutrient contents of leaf sample of banana plants.

Sample	Nutrients							
	[mg (g d.m.) ⁻¹]						[mg (kg d.m.) ⁻¹]	
	N	P	K	Ca	Mg	Fe	Zn	Mn
Banana leaf	2.82	0.19	3.85	1.4	0.46	119	21.7	163
Optimum levels (Lahav and Turner, 1983)	2.60	0.17-0.20	3.14-4.15	1.00	0.30	80.0	18.0	25.0
								6.4
								9.0

Table - 2 : The nutrient contents of compost and farmyard manure.

Sample	Plant nutrients								
	[mg (g d.m.) ⁻¹]					[mg (kg d.m.) ⁻¹]			
	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
Compost	2.94	0.6	4.2	1.9	0.8	95	114	206	8
Farmyard manure	2.56	0.7	4.6	7.5	1.3	422	247	319	21

pieces in the spring season. Approximately 25-30 cm of mixed banana chips were placed on a layer of coarse straw and stalk materials (15-20 cm in thick) lied on a solid and flat ground surface. The chips layer was covered by soil and then watered until run-off, respectively. Compost pile of 1.5 m in height was made by adding new layers one over another, covered with soil and washed by sprinkler until water was drained from bottom of pile, which was covered by a plastic sheet.

Compost pile was mixed once a month and periodically sprinkled with water to keep it moist for homogeneity of decomposition and speed up the maturing. The well-composted materials were separated during the monitoring of the process, and unmaturing materials were separately piled up and composted as described above. The process was completed within 5 months. Farmyard manure was prepared through heat fermentation of cattle manure (Özbek, 1981).

The compost and farmyard manure samples were analysed to determine their nutrient levels according to the above mentioned methods. The analysis results of compost and farmyard manure are given in Table 2.

While contents of N, P, K and Mg in compost and farmyard manure were found to be similar, Ca content of farmyard manure especially were found higher than compost. The ratio among nutrient levels of compost was more suitable than farmyard manure in terms of a balanced nutrition.

In the beginning, the leaf samples from the plants in each parcel were taken to determine the effects of different fertilizers on nutrient levels of leaves, in November 1995 and 1996.

Results and Discussion

The experimental soil used had a loamy texture (23.4% clay, 41.8% silt, 34.8% sand), a high

lime content, pH at low alkalinity, no salinity problem, the organic matter and available Zn contents at medium levels, the total N and available P, Fe, Mn, Cu and exchangeable K contents were at high levels (Table 3).

Since banana plants are more productive in soils which are rich in plant nutrients and humus, moderate acid in reaction, have no salinity problem, and are loose, deep, well aerated, sandy loamy-textured (Özbek, 1981; I.F.A., 1992), the experimental soil can be said to have been suitable for banana growing.

The experimental soil was rich in nutrients; therefore, the study had to be carried out after constituting poorer conditions in terms of nutrient contents in order to be able to determine the effects of different fertilizers on the nutrient contents in the leaves of banana plants. For that reason, banana plants were grown without application of any fertiliser in 1994.

The analysis results of leaf samples taken in both years of the experiment from the parcels of different fertilizers applied are given in Table 4.

As seen in Table 4, N, P and K contents of leaf samples in all applications except control, and Ca, Mg, Fe, Zn, Mn, Cu contents in all applications were determined between optimum levels of reference values given by Lahav and Turner (1983). When the two-years data were evaluated together, the effects of fertilizers applied on N, P, K, Ca and Mg contents of leaves were found significant while those of fertilizers applied on Fe, Zn, Mn and Cu contents were found insignificant. On the other hand Mn levels of this treatment plants were found much higher than the optimum levels of nutrients given by Lahav and Turner (1983). The effects of fertilizer types on nutrient contents of leaves according to LSD test evaluated are as seen in Table 4.

Table - 3 : Some physical and chemical properties of the soil.

Soil depth (cm)	Texture	pH	[mg (g) ⁻¹]			Available [mg (kg) ⁻¹]						
			Total soluble salt	Lime	Organic matter	Total N	P	K	Fe	Zn	Mn	Cu
0-30	Loamy	7.3	0.115	30	2.6	0.21	37	460	5.4	1.0	3.3	1.2
30-60	Loamy	7.7	0.069	32	3.4	0.28	21	210	8.8	0.8	4.1	1.1

Table - 4 : The effects of different fertilizers on the nutrient contents of leaves.

Treatments	Plant nutrients ¹									
	[mg (g d.m.) ⁻¹]					[mg (kg d.m.) ⁻¹]				
	N**	P**	K**	Ca**	Mg*	Fe	Zn	Mn	Cu	
Control	2.41 c	0.150 b	2.85 c	2.57 a	0.74 a	73	19	154	7	
15 kg plant ⁻¹ compost	2.72 ab	0.177 a	3.70 b	2.12 bc	0.60 ab	78	22	173	7	
30 kg plant ⁻¹ compost	2.84 ab	0.182 a	3.97 ab	1.88 bc	0.58 b	78	24	181	7	
45 kg plant ⁻¹ compost	2.86 ab	0.187 a	4.02 ab	1.90 bc	0.58 b	78	25	187	6	
Mineral fertilizers	2.70 b	0.182 a	3.85 ab	2.22 ab	0.61 ab	78	22	178	7	
50 kg plant ⁻¹ farmyard manure	2.93 a	0.193 a	4.16 a	1.78 c	0.46 b	84	25	164	8	
F.M. + M.F.	2.79 ab	0.193 a	4.10 a	2.06 bc	0.50 b	81	25	178	8	
LSD	0.207	0.024	0.333	0.428	0.148	n.s	n.s	n.s	n.s	

¹ Values are the average of two-year findings

n.s., *, **: Non-significant, significant at P=0.05, significant at P=0.01, respectively

a, b, c : Mean separation significant within column by LSD test.

those of the other nutrient contents were insignificant. While N, P, K, Fe, Mn and Cu contents of leaves in the first year of the study were found in higher levels, Ca, Mg and Zn contents of leaves were in higher levels in the second year. These findings can stem from high levels of lime, total N, P, K contents in the experimental soil and among nutrients synergistic effects in the first year and antagonistic effects in the second year.

While increasing doses of compost applications increased significantly the N, P and K contents of leaves, decreased the Ca and Mg contents of leaves. But Fe, Zn, Mn and Cu contents of leaves were not markedly affected by increasing doses of compost. Likewise Kaşka *et al.* (1991), found in a study conducted out that increasing levels of farmyard manure applications increased significantly the K, Ca and Zn contents of the leaves. But P, Mg, Mn and Cu contents of the leaves were also not affected by farmyard manure doses.

Due to improvement of nutrient levels through increasing rates of compost, it is estimated that nutrient contents would be increased more in a new study with compost doses above 45 kg plant⁻¹. The most favorable compost dose should be determined and used in banana fertilization hereafter.

Nitrogen : According to two-year average findings in Table 4, in view of fertiliser types applied, the average N contents of leaves ranked between 2.93% (50 kg plant⁻¹ F. M.) and 2.47% (control). The highest value was obtained from farmyard manure (2.93%), 45 kg plant⁻¹ of compost (2.86%) and 30 kg plant⁻¹ of compost (2.84%) ranged as second and third.

Lahav and Turner (1983) and Langenegger and Smith (1988) reported that optimum N levels of banana leaves should be between 2.5% and 3.0%. Also Warner and Fox (1977) reported that the nitrogen content of leaves should be 2.88% for high yield in banana plants

In other studies, Kaşka *et al.* (1991) reported that the leaf N content of leaves in farmyard manure applications ranked between 2.35% and 2.88%; Köseoğlu *et al.* (1985) found it as 2.36%-3.04%, and according to Özbek and Danişman (1978) the leaf N content of leaves was between 1.85% and 2.88%.

Phosphorus : The phosphorus contents of leaves ranked between 0.193% (50 kg plant⁻¹ F.M. and farmyard manure + mineral fertilizers) and 0.150% (control). The phosphorus content of leaves was attained the highest value (0.193 %) in farmyard manure and in farmyard manure + mineral fertilizers and 45 kg plant⁻¹ of compost followed these fertilizers with the value of 0.187%.

De Geus (1967) reported that the phosphorus content of leaves should be 0.21% for high yield in banana plants, likewise Lahav and Turner (1983) reported that minimum level of phosphorus content of leaves should be 0.17%.

In another studies, Kaşka *et al.* (1991) reported that the leaf P content of leaves in farmyard manure applications ranked between 0.26% and 0.27%; Köseoğlu *et al.* (1985) found it as 0.13%-0.25%, and according to Özbek and Danişman (1978) the leaf P content of leaves was between 0.13% and 0.18%.

Potassium : The average potassium contents of leaves ranked between 4.16% (50 kg plant⁻¹ F.M.) and 2.85% (control). The highest value was obtained from farmyard manure (4.16%), farmyard manure + mineral fertilizers (4.10%) and 45 kg plant⁻¹ of compost (4.02%) ranged as second and third.

According to Lahav and Turner (1983), minimum potassium content of leaves should be 3.14%, and also Turner and Barkus (1981) reported that this level was as 3.3%. Potassium levels of no potassium deficiency symptom showing plants were determined as 2.7% by Hewitt (1955). Also De Geus (1967) determined in a conducted study that high yield in banana plants was attained when potassium content of leaves was 3.27%. According to Fernandez and Fox (1985), minimum K content of leaves for a good growth and yield should be less than 3.2%.

In other studies, Kaşka *et al.* (1991) reported that the leaf K content of leaves in farmyard manure applications ranked between 3.60% and 4.63%; according to Köseoğlu *et al.* (1985) the leaf K content of leaves was between 2.74% and 5.09%.

Our findings about leaves' N, P and K contents of this treatment are consistent with the findings of De Geus (1967), Özbek and Danişman (1978), Lahav and Turner (1983), Köseoğlu *et al.* (1987) and Kaşka *et al.* (1991).

Calcium : The average calcium contents of leaves ranked between 2.57% (control) and 1.78% (50 kg plant⁻¹ F. M.). The lowest calcium content of leaves was attained 1.78% in farmyard manure and it was followed by 30 kg plant⁻¹ of compost (1.86%) and 45 kg plant⁻¹ of compost (1.90%) levels. Hewitt (1955) and Lahav and Turner (1983) reported that the average calcium content of leaves should be 1.0%.

In another study, Kaşka *et al.* (1991) reported that the leaf Ca content of leaves in farmyard manure applications ranked between 1.78% and 2.75%; Köseoğlu *et al.* (1985) found it as 1.0%-3.2%, and according to Özbek and Danişman (1978) the leaf Ca content of leaves was between 2.21% and 3.94%.

Magnesium : The average magnesium contents of leaves ranked between 0.74% (control) and 0.46% (50 kg plant⁻¹ F. M.). The lowest value was obtained from farmyard manure (0.46%), farmyard manure + mineral fertilizers (0.50%) and 45 kg plant⁻¹ of compost (0.58%) ranged as second and third.

Lahav and Turner (1983) reported that the magnesium content of leaves should be 0.3%; Murray (1960) determined that magnesium levels of no magnesium deficiency symptom showing plants as 0.36%. Also De Geus (1967), reported that high yield in banana plants was attained when magnesium content of leaves was 0.31%.

In another study, Kaşka *et al.* (1991) reported that the leaf Mg content of leaves in farmyard manure applications ranked between 0.50% and 0.63%; Köseoğlu *et al.* (1985) found it as 0.23%-0.74%, and according to Özbek and Danişman (1978) the leaf Mg content of leaves was between 0.39% and 0.89%.

While our results on leaves Ca and Mg contents are consistent with the findings of Lahav and Turner (1983), Köseoğlu *et al.* (1987) and Kaşka *et al.* (1991), the calcium findings of Özbek and Danişman (1978) were higher than the others.

Micro-nutrients : While the average iron content of leaves in compost applications was found as 78 ppm very close optimum levels, zinc and manganese contents increased as parallel with compost doses and stayed over optimum levels. The copper contents of leaves in all applications were stayed below optimum levels. But, If copper levels (5.3-8.0 ppm) determined by Özbek and Danişman (1978), Kaşka *et al.* (1991), in our country conditions are considered as reference values, our copper findings can be accepted as optimum levels.

In other studies conducted with Dwarf Cavendish clone, Kaşka *et al.* (1991) reported that the leaf Zn content of leaves in farmyard manure applications varied between 23.7 and 31.5 ppm; Mn 68.2 and 81.9 ppm; Cu 5.8 and 7.3 ppm, and according to Özbek and Danişman (1978), the leaf Zn content of leaves varied between 9.5 and 16.0 ppm; Fe 69.0 and 94.0 ppm; Mn 105 and 234 ppm; Cu 5.3 and 8.0 ppm. Our findings are consistent with the relevant literature.

The relationships among nutrient contents of banana leaves : For the correlation analysis to determine relationships among macronutrient contents of leaf samples average values of two-year data was used.

According to statistical evaluations, while there were significant positive correlations between N and P ($r : 0.867^*$), N and K ($r : 0.919^{**}$), there were significant negative correlations between N and Ca ($r : -0.931^{**}$), N and Mg ($r : -0.892^{**}$). Again, while there was significant positive correlation between P and K ($r : 0.933^{**}$), there was significant negative correlations between P and Ca ($r : -0.810^*$). On the other hand, there were significant negative correlations between K and Ca ($r : -0.907^{**}$), K and Mg ($r : -0.938^{**}$).

These findings can stem from synergetic effects of increased levels of N, P and K ions in the soil solution as a result of application of increased doses of compost, and antagonistic effects on Ca and

Mg ions of levels increased in the soil solution of N, P and K.

Köseoğlu *et al.* (1987) found in a survey study that while there were significant positive relations between N and K, N and P, Ca and Mg contents of banana plants, negative relations between K and Ca, K and Mg contents were evident. Lahav and Turner (1983) in a study conducted out with organic and mineral fertilizers, found an antagonistic relation between K and Mg content of leaves higher than an antagonistic relation between K and Ca content of leaves. According to the findings of Köseoğlu *et al.* (1987) and Kaşka *et al.* (1991) there was a significant negative relation between K and Mg contents in the leaves of banana plants, and Kaşka *et al.* (1991) reported that while farmyard manure applications in comparison with mineral fertilizers applications made an increasing effect on K levels of leaves, farmyard manure applications made a decreasing effect on Ca and Mg levels of leaves.

The relationships among nutrients in the leaves growth, yield and quality : Correlation calculations were made to determine the levels of relationships among N, P and K contents of leaves and growth, yield and quality characteristics of banana plants.

According to correlation analysis; there were positive correlations between N content of leaves and all of the following : bunch yield ($r : 0.811^*$), trunk diameter ($r : 0.783^*$), finger diameter ($r : 0.768^*$), leaf number ($r : 0.921^{**}$). Similarly, there were positive correlations between P content of leaves and all of the following : bunch yield ($r : 0.820^*$), trunk diameter ($r : 0.895^{**}$), leaf number ($r : 0.915^{**}$), number of hand ($r : 0.767^*$), finger diameter ($r : 0.894^{**}$), and also there were positive correlations between K content of leaves and all of the following : bunch yield ($r : 0.881^{**}$), trunk diameter ($r : 0.907^{**}$), leaf number ($r : 0.954^{**}$), finger diameter ($r : 0.893^{**}$), number of hand ($r : 0.793^*$).

Hewitt and Osborne (1962) reported that there was a very high correlation between K content of leaves and bunch yield, and also Bhango and Karan (1962) reported that there was a positive correlation between nutrient contents of leaves and bunch yield. Meanwhile Messing (1981) determined

The effects of compost on the nutrient contents of banana.

that there was an important positive correlation between the N contents of leaves and bunch yield ($r : 0.920$).

Conclusion

The highest nutrient contents of leaves of Dwarf Cavendish clone were obtained from farmyard manure. Farmyard manure + mineral fertilizers and 45 kg plant⁻¹ of compost ranged as second and third in nutrient contents of leaves.

While N, P and K contents of leaves in all applications except control were determined between optimum levels of reference values, Ca, Mg, Fe, Zn, Mn and Cu contents were between optimum levels of reference values in all applications.

The increasing doses of compost increased leaf nutrient contents of banana plants and 45 kg plant⁻¹ of compost was the most effective application among compost doses used.

Also Paydaş *et al.* (1988), found in a study conducted out that while P, Ca, Mg, Zn, Mn and Cu contents of the leaves were not markedly affected by various levels of N applications, and increasing levels of applied N decreased the N and K contents of the leaves. On the other hand, increasing levels of farmyard manure applications increased significantly the K, Ca and Zn contents of the leaves in comparison to controls. But P, Mg, Mn and Cu contents of the leaves were also not affected by farmyard manure applications, whereas the N levels in the leaves of farmyard manure were found higher than those of N applications.

Physical qualities of grown soil bananas are more important than chemical composition, because the roots are fragile and a great need of oxygen. Organic fertilizers are excellent for improving soil conditions and provide variable amounts of nutrients. Therefore, farmyard manure and compost are widely used for decades in many countries (I. F. A., 1992).

In banana, production is needed in higher amount to potassium and nitrogen among macro nutrients and is most important K : N ratio for optimum yield, and K absorption is largest during bunch growth; N and P continuous uptake from

planting to bunch emergence (Özbek, 1981; Martin-Prevel *et al.*, 1987; I. F. A., 1992).

Balance farmyard manure + mineral fertilizers or compost adequate in nutrients may also supply physical qualities of soil and nutrients needed for banana production.

When results were compared with those obtained by utilizing organic and inorganic fertilizers, the use of the 45 kg plant⁻¹ of banana compost was determined to be more suitable in terms of economical production and organic farming than other fertiliser applications.

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Effect of fenvalerate technical grade on acetyl cholinesterase activity in Indian bullfrog *Haplobatrachus tigerinus* (Daudin)

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Abstract : Indian bull frog *Haplobatrachus tigerinus* (Daudin) was exposed to sublethal dose (1/3 of LC_{50} I.E. 1.166 mg/kg) of fenvalerate technical grade and the effect was studied on the specific activity of acetyl cholinesterase in the different tissues of frog viz., brain, muscle, liver, kidney and testis at different time periods viz., 3, 6, 12, 24, 48 and 72 hours. The inhibition of specific activity of acetyl cholinesterase was in the order of kidney > brain > muscle > liver > testis. A significant inhibition was noticed in kidney at 12 hours (-64.33%) and no effect was noticed at 3 hours in testis (+0.67%). The AChE activity was inhibited in first three hours of administration of fenvalerate in all the tissue tested. The inhibition continued upto 6 hours or 2 hours in different tissue but the recovery was started by 24 hours and almost completed by 72 hours.

Key words : Acetyl cholinesterase, Non-target species, Fenvalerate.

Introduction

Pyrethroids are typical neurotoxic compounds structurally related to the natural pyrethrins, which have been synthesized during the last few decades for their excellent insecticidal properties (Elliot and Janes, 1978; Naumann, 1981). The pesticides, which are neurotoxic, are known to cause acute toxic effects in man and animals, the signs and symptoms of which are those of excess acetylcholine due to irreversible inhibition of AChE. Natural pyrethroids and allethrin are known to stimulate and subsequently paralyse various nerves (Camougis, 1973; Narahasi, 1971). Cypermethrin and fenvalerate were found to have inhibitory effect on serum acetyl cholinesterase activity of cattle and buffalo infested with *B. microplus* (Ansari *et al.*, 1989).

The primary site of action of pyrethroids may be influenced by a large number of factors including penetration, distribution and selective accumulation in tissue, biotransformation and elimination (O'Brien, 1967; Narahasi, 1971; Brooks, 1976). Acetyl cholinesterase terminates the action of acetylcholine in the nervous system (Taylor, 1985; Karczmer, 1970). The increase of acetylcholine at cholinergic synapses resulting from the inhibition of AChE, particularly in brain and diaphragm,

produces a variety of pharmacological effects that culminate in death by respiratory failure (Natcoff and Reiff, 1970).

Fenvalerate (RS) α -cyano-3-phenoxy-benzyl (RS) -2- (4 Chlorophenyl) -3-methyl -butyrate is an effective synthetic pyrethroid insecticide possessing excellent insecticidal activity and favourable environmental stability (Bradbury and Coats, 1982). Frogs constitute a very important group of animals that has been utilised by man very extensively. The reports on the effect of synthetic pyrethroid fenvalerate on AChE activity levels of frogs are very few. Hence, the present study of AChE, activity level in different tissue of Indian bull frog *Haplobatrachus tigerinus* (Daudin) following the treatment of synthetic pyrethroid, technical grade fenvalerate was attempted.

Materials and Methods

Frog *Haplobatrachus tigerinus* (Daudin) of size 10.6 ± 0.2 cm and 100-110 gram weight were brought from in and around Guntur and Vijayawada (Andhra Pradesh, India) and acclimatized at 25-30°C in the laboratory for 4 days. They were fed with cockroaches, earthworm and with small frogs and were tested for residue analysis. The feeding was stopped one day prior to the experiment. Such

acclimatized frogs were exposed to sublethal dose of fenvalerate technical grade (LD_{50} 3.5 mg/kg. sublethal dose = $1/3$ of LD_{50} I.E. 1.166 mg/kg). Technical grade fenvalerate (93.7% w/v) was supplied by Gujarat pesticides, Ahmedabad, Gujarat state, India. AChE assays were performed spectrophotometrically with the method of Ellman *et al.* (1961).

Results and Discussion

The AChE activity estimated in different tissues like muscle, liver, kidney, testis and brain in frog *H. tigerinus* (Daudin) treated with technical grade fenvalerate after 3, 6, 12, 24, 48 and 72 hours

and the values along with standard deviation and percent change over control are shown in Fig. 1 and maximum inhibition of the enzyme and recovery timings were shown in Table 1.

In the present study, the AChE activity was inhibited in first three hours of administration of fenvalerate in all the tissue tested. The inhibition continued upto 6 or 12 hours in different tissues but the recovery was started by 24 hours and almost completed by 72 hours.

In brain tissue, the maximum inhibition of AChE activity was noticed at 6 hours. From 12 hours onwards, the recovery started and continued in

Table - 1 : Acetylcholinesterase inhibitor and recovery times of the frog *H. tigerinus* (Daudin) under sublethal exposure to fenvalerate technical grade.

Tissue	Maximum inhibition in hours	Recovery to normal condition in hours
1) Brain	0-6	12-72
2) Muscle	3-6	12-72
3) Liver	3-6-12	24-72
4) Kidney	0-12	24-72
5) Testis	--	--

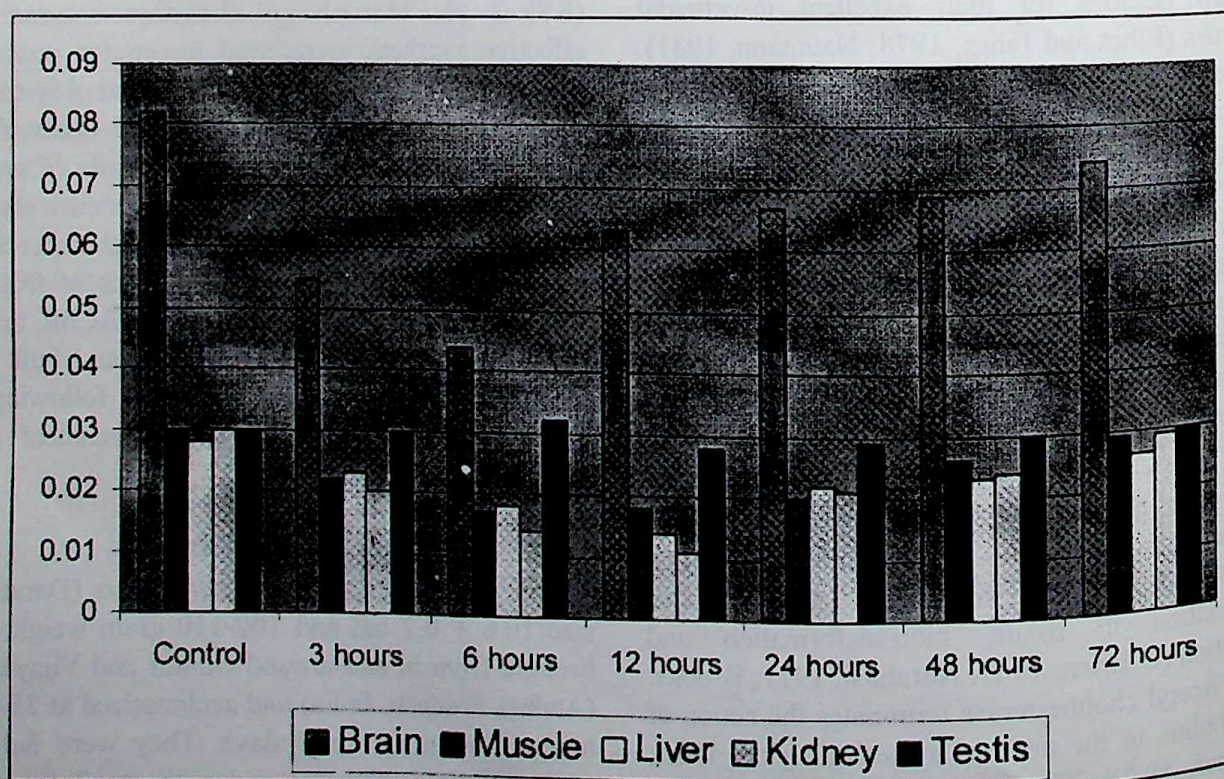


Fig. 1 : Effect of fenvalerate on the specific activity of acetylcholinesterase (AChE) (μ moles of acetylcholine hydrolyzed / mg / protein / min) in the different tissues of *H. tigerinus* (Daudin) at different time periods after injection of sublethal dose.

Effect of fenvalerate on acetyl cholinesterase activity.

the consecutive test, periods and not completed by 72 hours and the inhibition was 9.75% only over control.

In muscle tissue, the enzyme inhibition increased from 3 hours to 6 hours and maximum was at 6 hours. The recovery started from 12 hours exposure and was almost complete by 72 hours.

At 12 hours time period, the enzyme inhibition over control was 4.69% and the inhibition was only -1.34% at 24 hours; however there was very slight increase at 48 hours and 72 hours which were not significant. Narahasi (1971) stated that pyrethroids could be degraded by vertebrates with specific degrading mechanism, which supports the present study. In the present study, the degradation of pyrethroid fenvalerate resulted in the reversal of enzyme activity to almost normal level by 72 hours.

Cholinesterase is an important enzyme in toxicology. There are two types of cholinesterases; one is called acetyl cholinesterase or true cholinesterase, which occurs, in vertebrate erythrocytes and the electric organ of electric eels and fishes. This enzyme is the target of organophosphate and other neuro toxicants. The second one is called pseudo-cholinesterase or plasma-cholinesterase. The brain AChE activity was highest in chicken followed by rat and frog with acute intraperitoneal administration of organo-phosphates (Chattopadhyay *et al.*, 1986). Frog brain AChE was least sensitive to organophosphorus compounds (Edery and Schatzberg-porath, 1960; Tucker and Crabtree, 1970; Williams *et al.*, 1984).

Acetyl cholinesterase is involved in the maintenance of the structural and functional integrity of cellular membrane. The action of this enzyme and metabolism of individual groups of compounds were reviewed by O'Brien (1960). The extent of brain AChE reduction was proportional to the concentration of the substance. Acetyl cholinesterase activity in nerve endings of tails of *H. tigerinus* (Daudin) and *R. catesbeiana* was studied by Sasaki *et al.* (1985). Cypermethrin and fenvalerate were found to have inhibitory effect on serum cholinesterase activity of cattle and buffalo (Ansari *et al.*, 1989).

The increase in brain AChE level was greater at 10 minutes after exposure to propoxur to mice and by 60 minutes the recovery started (Kobayashi, 1988). Monocrotophos in all doses (10 to 40 mg/kg body weight) inhibited erythrocyte cholinesterase from 12 to 48 hours of administration in buffalo calves (Sandhu and Malik, 1988).

In conclusion, the insecticides as fenvalerate as well as other pesticides are known to be highly hazardous to non-target organisms, particularly the amphibian population, as they feed on insects are more often affected. As the frogs are involved in pest control, it is hard to believe that the frog leg meat consumption is solely responsible for an upsurge of vectors and pests. The non-edible species of frogs and toads became equally important from the point of view of pest control. Further, the depletion in the anuran population is attributable to the indiscriminate use of pesticides (Mohanty-hejmadi and Dutta, 1981). Hence, the need to protect the valuable frogs from undue exposure of insecticides cannot be ignored.

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Effect of sodium metabisulphite on germination, growth and yield of *Vigna sinensis*, Savi

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Abstract : In the present study, the impact of sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$), a food preservative, on seed germination, growth and yield of *Vigna sinensis*, Savi has been evaluated. Observations clearly reveal the deleterious effect of $\text{Na}_2\text{S}_2\text{O}_5$ on germination, stomatal development, stomatal index, chlorophyll content and yield. The shoot length exhibited a steady rise in length, while the biomass showed a gradual decrease with the increasing doses of $\text{Na}_2\text{S}_2\text{O}_5$.

Key words : Sodium metabisulphite, *Vigna sinensis*, Germination, Growth, Yield.

Introduction

The management of environment has attracted international attention in current years. Due to technological development and urbanization, a number of undesirable substances are dumped into the surroundings, of which majority are labelled as environmental mutagens. In the recent years, we have been witnessing a rapid growth of food industry. Food additives are substances added to the food to make it more attractive. Some of the commonly used food preservatives are sodium metabisulphite, potassium metabisulphite and formaldehyde. Mutagenic action of sodium metabisulphite was revealed by the works of Hayatsu and Miura (1970) in *E. coli*. The genotoxic effect of sodium metabisulphite was further proved in mouse *in vivo* test system by Pal and Bhunya (1992). Higher plants offer many advantages for screening and monitoring of environmental chemicals for their potential mutagenicity. The present work was undertaken to investigate the effects of sodium metabisulphite on germination, stomatal development, stomatal structure and aberration, chlorophyll content and biomass of *Vigna sinensis*.

Materials and Methods

Seeds of *Vigna sinensis*, Savi were selected as experimental material. For germination studies, about 10 good quality seeds were taken in separate sterilized petridish lined with Whatman No.1 filter

paper. Sodium metabisulphite solutions (1 $\mu\text{g/ml}$, 2 $\mu\text{g/ml}$, 3 $\mu\text{g/ml}$, 4 $\mu\text{g/ml}$ and 5 $\mu\text{g/ml}$) were prepared in distilled water. Moistening of the filter paper was done with the respective solutions. Distilled water was used for the control. Three replicates for each treatment were kept at room temperature. Recording was done at every 24 hours. The percentage of germination was expressed based on the number of seedlings emerged out of the total number of seeds tried for germination. The development of stomata was studied using a light microscope.

For the pot culture experiments, pre-soaked seeds were sown at a depth of 1 inch in earthenware pots filled with garden soil mixed with cow dung. Pots were watered with different concentrations of $\text{Na}_2\text{S}_2\text{O}_5$ and the control sets were watered with distilled water. All the experiments were raised in triplicates. Treatments started when the plants were 14 days old. Plants were analysed for various parameters like structure and development of stomata, stomatal index, chlorophyll content (Aron, 1949), length of plants, yield and fresh and dry weights.

Results and Discussion

Observations conducted on various parameters clearly indicated that sodium metabisulphite has an inhibitory effect on *Vigna sinensis*, Savi. Germination of seeds was greatly influenced by the treatment with $\text{Na}_2\text{S}_2\text{O}_5$. Cent

percent germination was recorded in the control and the germination (%) decreased gradually with the increase of $\text{Na}_2\text{S}_2\text{O}_5$ concentration, maximum being for the treatment with 5 $\mu\text{g/ml}$ $\text{Na}_2\text{S}_2\text{O}_5$ (65%, Table-1). In addition, the emergence of radicle and plumule was delayed with the (65%, Table-1). In addition, the emergence of radicle and plumule was delayed with the increasing concentration of $\text{Na}_2\text{S}_2\text{O}_5$. This is in accordance with the findings of Mehta and Desai (1958) and Bhumbia and Singh (1965).

The epidermal cells and stomata were found to be normal in untreated seedlings (Fig. 1A). Division of stomatal initial and the formation of guard cells showed abnormalities in the plants with $\text{Na}_2\text{S}_2\text{O}_5$ treatments. The plants exposed to higher concentrations of $\text{Na}_2\text{S}_2\text{O}_5$ showed aberrations like 1) stoma with single guard cell, 2) arrested development, and 3) degeneration of guard cell. Though, the response of foliar epidermis to diverse types and levels of air pollution (Ramanathan and Kanabiran, 1989; Farouqi and Singh, 1990; Sathyanarayana *et al.*, 1990; Salgare and Acharekar, 1991) and water pollutants like heavy metals (Srivastava and Baniskar, 1996; Yadav and Srivastava, 2000 and Bindhu and Bera, 2001) has been analysed and observed the modifications in the epidermal and stomatal features, the exploration of the effect of food preservative $\text{Na}_2\text{S}_2\text{O}_5$ on this characteristic has not probably been undertaken so far.

Single guard cells were observed in treatments with 2 $\mu\text{g/ml}$, 3 $\mu\text{g/ml}$, 4 $\mu\text{g/ml}$ and 5 $\mu\text{g/ml}$ $\text{Na}_2\text{S}_2\text{O}_5$ (Fig. 1 B, C, D and E). According to Inamdar (1970), Patel and Shah (1971) and Patel *et al.* (1972), single guard cells are formed either by the degeneration of one of the pair of guard cells of a normal mature stoma or by a developmental anomaly i.e., by the failure of the mother guard cell to divide further to produce a pair of guard cells. The stomatal index, which was 38 on the lower epidermis of a normal plant, showed a decreasing trend as the concentration of $\text{Na}_2\text{S}_2\text{O}_5$ increased, marked difference being noted in case of treatments with 3, 4 and 5 $\mu\text{g/ml}$ $\text{Na}_2\text{S}_2\text{O}_5$ (Table-1).

The decline in the chlorophyll content, yield and biomass of *Vigna sinensis* treated with different concentrations of $\text{Na}_2\text{S}_2\text{O}_5$ proved the deleterious effect of this food preservative on the growth and metabolism of the plant. The plants kept as control possessed the maximum chlorophyll content. The least amount of chlorophyll content was noted in case of treatment with 5 $\mu\text{g/ml}$ $\text{Na}_2\text{S}_2\text{O}_5$ and the reduction in the total chlorophyll was to the extent of 44.53% at 5 $\mu\text{g/ml}$ $\text{Na}_2\text{S}_2\text{O}_5$ (Table-1).

This shows that $\text{Na}_2\text{S}_2\text{O}_5$ exerted inhibition in the development of chlorophyll pigments in *Vigna sinensis*, Savi.

The maximum yield and total biomass were in the untreated plants and started declining with the increasing concentration of $\text{Na}_2\text{S}_2\text{O}_5$ (Table 1). The

Table - 1 : Effect of various concentrations of $\text{Na}_2\text{S}_2\text{O}_5$ on seed germination, stomatal index, shoot length, biomass chlorophyll content and yield of *Vigna sinensis*, Savi.

Concentration $\mu\text{g/ml}$	Control	1	2	3	4	5
Germination (%)	100	90	85	75	70	65
Stomatal index	38	35	33	28	15	10
Length of shoot (cm)	63.5	105	110.5	113	119	153
Fresh wt. (gm)	28.2	27.8	22.8	17.2	11.5	9
Dry wt. (gm)	6	4.5	3.5	3.5	1.7	1.6
Chl a mg/gm	0.5251	0.4585	0.4268	0.4007	0.3613	0.3224
% inhibition	0	12.68	18.72	23.69	31.19	38.6
Chl b mg/gm	1.2393	0.7512	0.710	0.6281	0.5241	0.4201
% inhibition	0	39.38	42.71	49.32	57.71	66.10
Total chlorophyll mg/gm	1.599	1.3043	1.2173	1.13	1.0087	0.8869
% inhibition	0	18.43	23.87	29.33	36.92	44.53
No. of fruits/plant	10	6	6	6	5	5

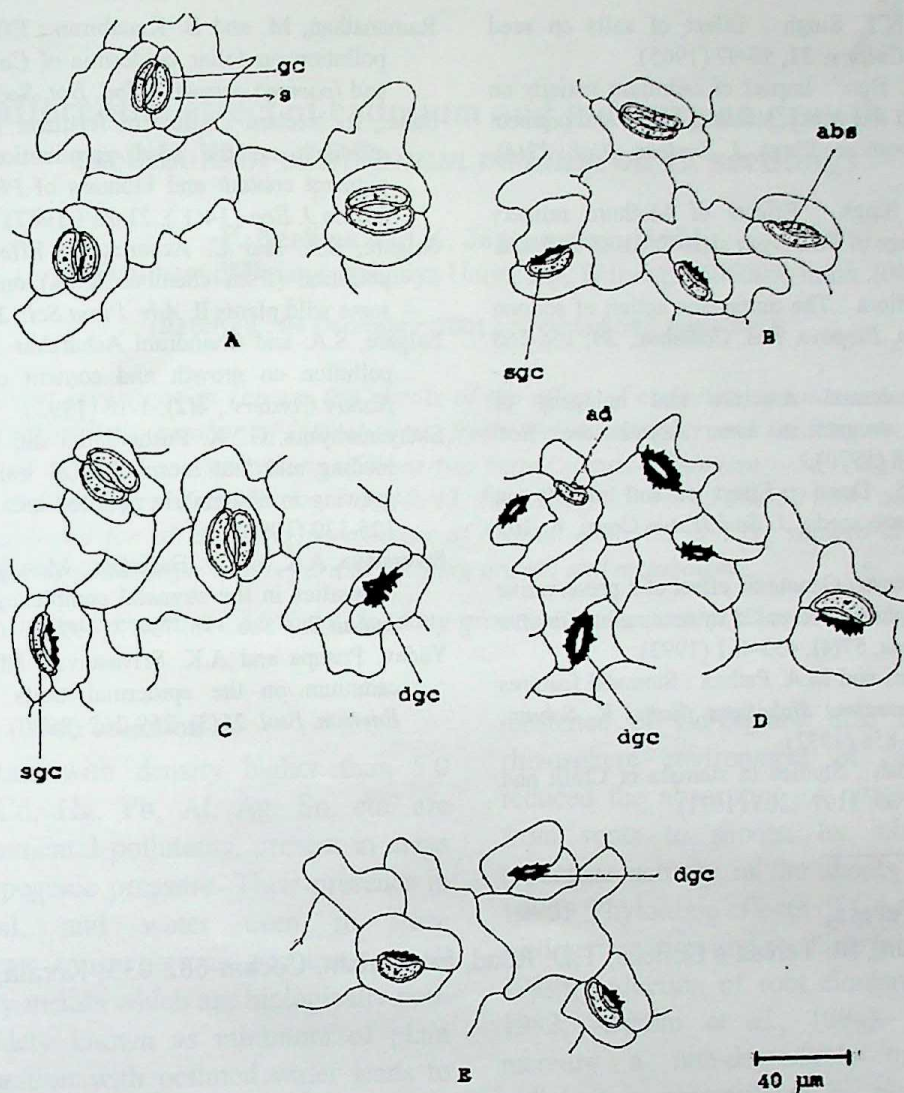
Sodium metabisulphite and germination of Vigna.

Fig. 1 : Epidermal peels of *Vigna sinensis*, Savi treated with sodium metabisulphite showing mature stomata and aberrations.

A - control; B - 2 µg/ml; C - 3 µg/ml; D - 4 µg/ml; E - 5 µg/ml; gc - guard cell;
 s - subsidiary cell; abs - abnormal stomata; sgc - single guard cell; dgc - degenerated guard cell;
 ad - arrested development

number of fruits/plant which was 10 in the control became 6 in the plants treated with 1, 2, 3 µg/ml $\text{Na}_2\text{S}_2\text{O}_5$ and it further declined to 5 fruits/plant in case of treatment with 4 and 5 µg/ml $\text{Na}_2\text{S}_2\text{O}_5$. From the findings, it is obvious that there exists a direct relationship between the pigment concentration and the yield and biomass. The present observations therefore agree with those of Sahai (1987) and Salgare and Acharekar Chandrani (1992).

With regard to the height, control plant exhibited the minimum length (63.5 cm) giving an almost rosette appearance while the maximum shoot

length (153 cm) was observed in plants exposed to 5 µg/ml $\text{Na}_2\text{S}_2\text{O}_5$ (Table-1). This might be due to the inducing effect of $\text{Na}_2\text{S}_2\text{O}_5$ on the elongation of internodes.

From the present data, it can be concluded that sodium metabisulphite has a detrimental effect on germination of *Vigna sinensis*, Savi.

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Differential effect of cadmium and mercury on growth and metabolism of *Solanum melongena* L. seedlings

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Abstract : The present investigation reports the results of the effect of cadmium and mercury individually on seed germination, seedling growth, number of lateral roots, fresh and dry weights and seedling metabolism in *Solanum melongena*. Effect of different concentrations of these two heavy metals (Cadmium – 50, 100, 300, 500, 700, 1000, 3000, 5000, 7000 and 9000 ppm and Mercury – 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 ppm) and durations for 6, 12 and 24 h were employed for all seedling parameters of brinjal. Both Cd and Hg showed drastic effects at high concentrations and longer duration with regard to seedling growth and metabolism.

Key words : Heavy metals, Cadmium, Mercury, Seedling growth, Protein content, Protease activity, Amylase activity, *Solanum melongena*.

Introduction

The metals with density higher than 5.0 g/cm³ such as Cd, Hg, Pb, Al, Ag, Sn, etc. are important environmental pollutants, present in areas with high anthropogenic pressure. Their presence in atmosphere, soil, and water even in trace concentrations can cause serious problems to all organisms. Heavy metals which are biologically non-essential are widely known as inhibitors of plant metabolism. Irrigation with polluted water leads to heavy metal accumulation not only in the soil but also in plant parts including seeds (Banerji and Kumar, 1979). Excess Cd causes a number of toxic symptoms in plants i.e., growth retardation, inhibition of photosynthesis. Cadmium pollution is increasing in the environment due to mining, industrial usage and many anthropogenic activities. Cd released into the environment may affect human health (itai-itai disease is caused by Cd-contaminated rice in Japan). The acute toxicity of Cd compounds may affect various plant physiological processes such as seedling growth (Snehlata, 1983), gaseous exchange (Bazzaz *et al.*, 1979), photosynthesis (Roger *et al.*, 1975) and nutrient uptake and distribution (Oberlander and Roth, 1978). Recently Rashmi Nigam *et al.* (2002) reported significant increase in Cd accumulation in various plant tissues of *Zea mays* with increasing supplementation of phytochelators (organic acids) and suggested the

existence of Cd-organic acid interaction in soil-rhizosphere environment of the plant. Cd also reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting the nitrate reductase activity in the shoots (Hernandez *et al.*, 1990). Phytotoxic effects of Cd include inhibition of seed germination and seedling growth (Renjini *et al.*, 1989), reduction of root elongation (Rascio *et al.*, 1993; Arduini *et al.*, 1994). In polluted areas, mercury a non-degradable toxic heavy metal pollutant is accumulated by plants. Mukherji and Ganguly (1974) have observed the toxic effect of mercury on germinating rice seeds and on physiological aspects of several legumes (Sharma, 1982). Mishra and Choudhuri (1997) have reported the differential effects of Pb²⁺ and Hg²⁺ on inhibition of germination of seeds of two rice cultivars. The present work aims to evaluate the effect caused by Cd and Hg on seedling growth, number of lateral roots, fresh and dry weights and seedling metabolism in *Solanum melongena*.

Materials and Methods

Healthy seeds of *S. melongena* cv PPR selected for uniformity and allow to imbibe in water for 2 h and were surface sterilized with 0.1% HgCl₂ and then washed with distilled water. There was no additive effect with seed surface sterilization on the doses of HgCl₂ used. After washing thoroughly these

were soaked in different concentrations of Cadmium acetate (50, 100, 300, 500, 700, 1000, 3000, 5000, 7000 and 9000 ppm) and Mercuric chloride (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 ppm) solutions for different durations of 6, 12 and 24 h respectively. Particularly for Cd treatment seed germination was tested even upto 20,000 ppm. Seeds soaked in distilled water were constituted as controls. After treatment, seeds were washed thoroughly and

transferred to wet Whatman no. 1 filter paper contained in petriplates for seedling growth in laboratory conditions. Controls were also maintained simultaneously. The percentage of germination was considered after radicle emergence. The seedling growth was studied after 15 days of planting. Fresh and dry weights of the seedlings were also calculated. For dry weight analysis seedlings were kept in oven for 48 h at 60°C. All determinations

Table - 1 : Effect of cadmium on germination and seedling growth of *Solanum melongena* after 15 days of planting.

Duration	Concentrations	% Germination	Shoot length	Root length	Total length	Number of lateral roots
6h	Control	90	3.6± 1.44	2.3± 0.588	5.9± 2.02	---
	50	50	2.92 ± 1.699	*3.0± 1.794	5.92 ± 3.493	0.5± 0.049
	100	50	2.6± 1.347	2.38 ± 1.129	4.98 ± 2.476	0.33 ± 0.022
	300	60	2.35 ± 0.92	2.3± 0.881	4.65 ± 1.801	0.14 ± 0.003
	500	65	1.66 ± 0.424	*1.86 ± 0.532	3.52 ± 0.956	0.11 ± 0.002
	700	70	1.5± 0.320	1.5± 0.320	3.0± 0.640	---
	1000	80	1.38 ± 0.238	1.32 ± 0.218	2.70 ± 0.456	---
	3000	85	1.29 ± 0.192	1.25 ± 0.181	2.54 ± 0.373	---
	5000	85	1.24 ± 0.178	1.2± 0.166	2.44 ± 0.344	---
	7000	90	1.19 ± 0.157	1.12 ± 0.139	2.31 ± 0.296	---
	9000	90	1.17 ± 0.152	1.1± 0.134	2.27 ± 0.286	---
12h	Control	90	3.5± 1.361	2.5± 0.694	6.0± 2.055	---
	50	50	2.86 ± 1.63	2.45 ± 1.196	5.31 ± 2.826	0.22 ± 0.0096
	100	55	2.54 ± 1.168	2.38 ± 1.026	4.92 ± 2.194	0.16 ± 0.0046
	300	60	2.22 ± 0.821	2.38 ± 0.944	4.60 ± 1.765	0.1± 0.0017
	500	65	1.66 ± 0.424	1.52 ± 0.355	3.18 ± 0.779	---
	700	65	1.31 ± 0.264	1.29 ± 0.256	2.60 ± 0.52	---
	1000	70	1.28 ± 0.233	1.25 ± 0.222	2.53 ± 0.455	---
	3000	75	1.24 ± 0.205	1.2± 0.192	2.44 ± 0.397	---
	5000	75	1.19 ± 0.189	1.15 ± 0.176	2.34 ± 0.365	---
	7000	80	1.17 ± 0.171	1.11 ± 0.154	2.28 ± 0.325	---
	9000	80	1.12 ± 0.157	0.8± 0.079	1.92 ± 0.236	---
24h	Control	80	2.9± 1.05	2.3± 0.661	5.2 ± 1.711	---
	50	40	2.75 ± 1.891	2.28 ± 1.299	5.03 ± 3.19	0.2± 0.01
	100	45	2.5± 1.391	2.21 ± 1.087	4.71 ± 2.478	0.14 ± 0.004
	300	45	2.11 ± 0.991	2.09 ± 0.972	4.20 ± 1.963	0.1± 0.002
	500	50	1.82 ± 0.66	1.65 ± 0.543	3.47 ± 1.203	---
	700	60	1.25 ± 0.260	1.21 ± 0.244	2.46 ± 0.504	---
	1000	60	1.19 ± 0.234	1.14 ± 0.216	2.33 ± 0.45	---
	3000	65	1.13 ± 0.196	1.11 ± 0.189	2.24 ± 0.385	---
	5000	70	1.11 ± 0.175	1.11 ± 0.175	2.22 ± 0.350	---
	7000	70	1.02 ± 0.148	0.92 ± 1.121	1.94 ± 0.269	---
	9000	70	*0.8± 0.091	*0.51 ± 0.37	1.31 ± 0.461	---

*Significant at 1% level

Mean ± S.E.

were carried out in triplicate and data analyzed statistically. The significance was calculated on the basis of chi-square test.

Results and Discussion

Table 1 and 2 shows the effect of different concentrations of Cd and Hg individually on seedling growth of *S. melongena*. Cd and Hg inhibit growth of seedling as their concentrations increase. However, in Cd treatment there was no effect on

germination. As the concentration increases, the germination percentage was also increased in all durations of Cd. But in Hg it was inversely correlated in all durations as concentration increases. Hence, the effect of Cd on seed germination (emergence of radicle) was insignificant even upto 20,000 ppm, however curling and stunted growth pronounced as increase in concentrations and durations. The effect of Hg inhibited seed germination significantly, compared to controls.

Table - 2 : Effect of mercury on germination and seedling growth of *Solanum melongena* after 15 days of planting

Duration	Concentrations	% Germination	Shoot length	Root length	Total length	Number of lateral roots
6h	Control	90	3.6 ± 1.44	2.3 ± 0.588	5.9 ± 2.02	—
	5	90	2.86 ± 0.909	2.3 ± 0.588	5.16 ± 1.497	0.25 ± 0.007
	10	80	2.65 ± 0.877	2.28 ± 0.649	4.93 ± 1.526	0.19 ± 0.005
	15	80	2.51 ± 0.787	2.25 ± 0.632	4.76 ± 1.419	0.14 ± 0.0024
	20	75	2.5 ± 0.832	2.19 ± 0.639	4.69 ± 1.471	—
	25	70	2.42 ± 0.834	2.15 ± 0.658	4.57 ± 1.492	—
	30	70	2.17 ± 0.671	2.10 ± 0.628	4.27 ± 1.299	—
	35	65	1.95 ± 0.585	1.85 ± 0.526	3.80 ± 1.111	—
	40	60	1.87 ± 0.583	1.81 ± 0.546	3.68 ± 1.129	—
	45	50	1.82 ± 0.66	0.98 ± 0.191	2.80 ± 0.851	—
	50	40	1.8 ± 0.81	0.67 ± 0.112	2.47 ± 0.922	—
12h	Control	90	3.5 ± 1.361	2.5 ± 0.694	6.0 ± 2.055	—
	5	80	2.25 ± 0.632	2.28 ± 0.649	4.53 ± 1.281	0.23 ± 0.0066
	10	80	2.19 ± 0.599	2.20 ± 0.604	4.39 ± 1.203	0.15 ± 0.0028
	15	75	2.1 ± 0.587	2.02 ± 0.543	4.12 ± 1.13	0.1 ± 0.0013
	20	70	2.04 ± 0.593	1.93 ± 0.530	3.97 ± 1.123	—
	25	70	1.75 ± 0.436	1.68 ± 0.402	3.43 ± 0.838	—
	30	65	1.65 ± 0.419	1.57 ± 0.379	3.22 ± 0.798	—
	35	60	1.59 ± 0.421	1.51 ± 0.379	3.10 ± 0.8	—
	40	50	1.42 ± 0.402	1.35 ± 0.363	2.77 ± 0.765	—
	45	40	1.35 ± 0.456	0.87 ± 0.189	2.22 ± 0.645	—
	50	30	1.28 ± 0.546	$*0.6 \pm 0.12$	1.88 ± 0.666	—
24h	Control	80	2.9 ± 1.05	2.3 ± 0.661	5.2 ± 1.711	—
	5	70	2.12 ± 0.64	2.08 ± 0.616	4.20 ± 1.256	0.19 ± 0.005
	10	65	2.05 ± 0.646	1.96 ± 0.591	4.01 ± 1.237	0.12 ± 0.0022
	15	65	1.97 ± 0.597	1.75 ± 0.471	3.72 ± 1.068	0.09 ± 0.0012
	20	60	1.78 ± 0.528	1.57 ± 0.411	3.35 ± 0.939	—
	25	60	1.59 ± 0.421	1.46 ± 0.355	3.05 ± 0.776	—
	30	50	1.45 ± 0.419	1.39 ± 2.84	2.84 ± 0.804	—
	35	40	1.41 ± 0.497	1.28 ± 0.409	2.69 ± 0.906	—
	40	30	1.27 ± 0.538	1.15 ± 0.441	2.42 ± 0.979	—
	45	30	1.18 ± 0.464	1.02 ± 0.347	2.20 ± 0.811	—
	50	20	$*3.4 \pm 5.782$	$*6.5 \pm 21.13$	9.9 ± 26.912	—

*Significant at 1% level

Mean \pm S.E.

The inhibitory effect on growth of seedling was more pronounced at higher concentrations of both metals. Shoot length and root length of seedlings decreased with increasing concentrations and durations for both metal treatments. However, in

HgCl_2 treatment the shoot length and root length at 50 ppm treated for 24 h was increased significantly. Lateral roots were not seen in control samples whereas in some of the treated samples they were clearly induced. Insignificantly the number of lateral

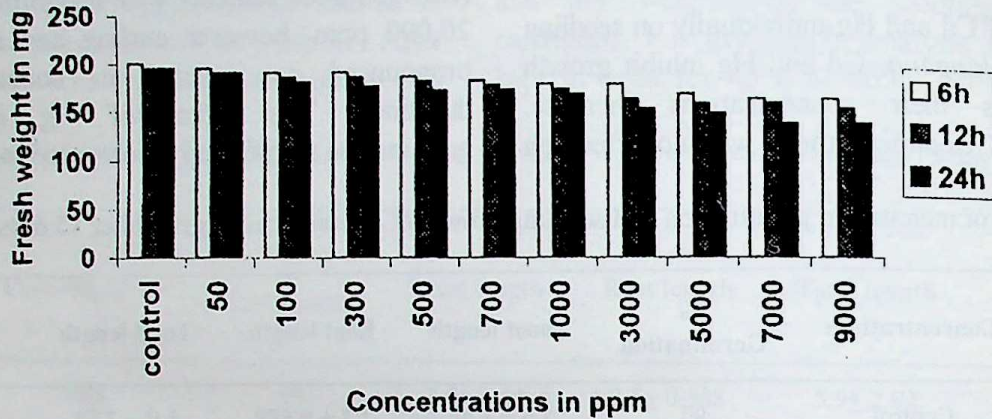


Fig. 1 : Effect of Cd on fresh weight in *S. melongena*.

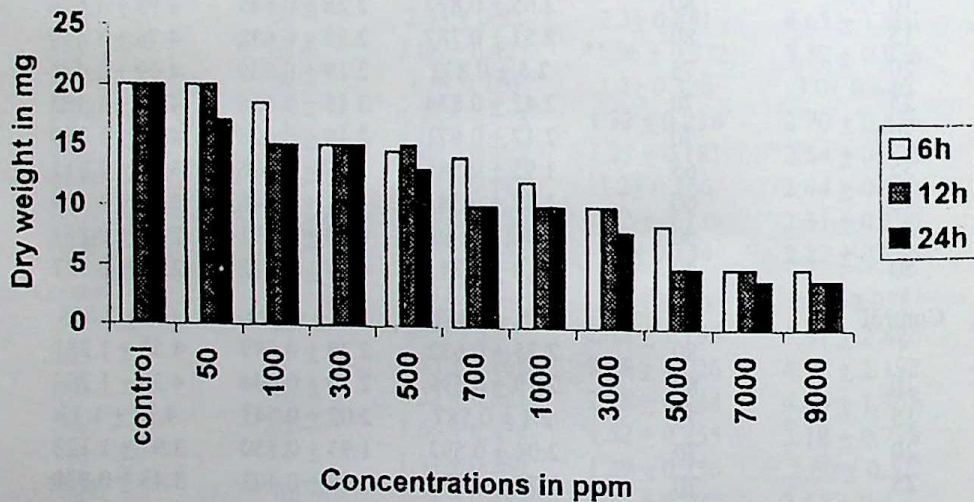


Fig. 2 : Effect of Cd on dry weight in *S. melongena*.

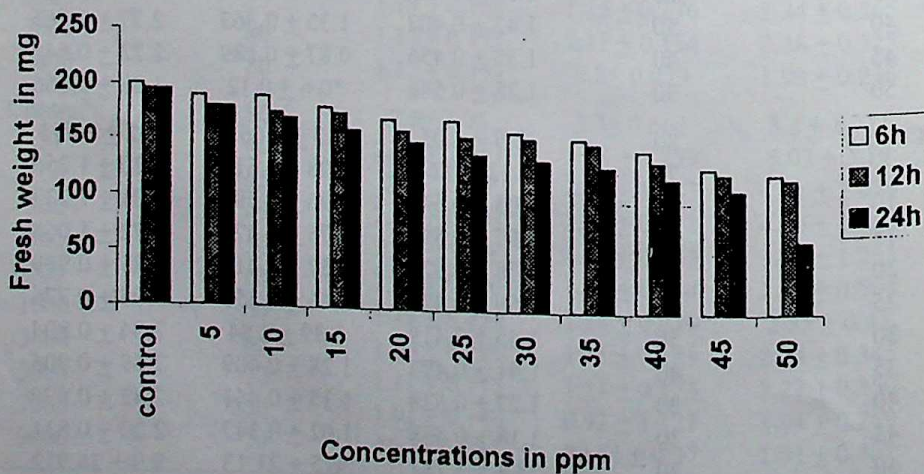


Fig. 3 : Effect of Hg on fresh weight in *S. melongena*.

roots induced in lower treatments (50-500 ppm) of Cd for all durations. The similar inhibitory effect on inducement of lateral roots was also observed in Hg treatment in lower concentrations (5, 10 and 15 ppm). The effect of Cd and Hg on fresh and dry

weights of seedlings correlated with the growth of the seedlings (Figs. 1-4).

Some biochemical estimations were carried out after 48 h of seed germination. The data are represented in Figs. 5-10.

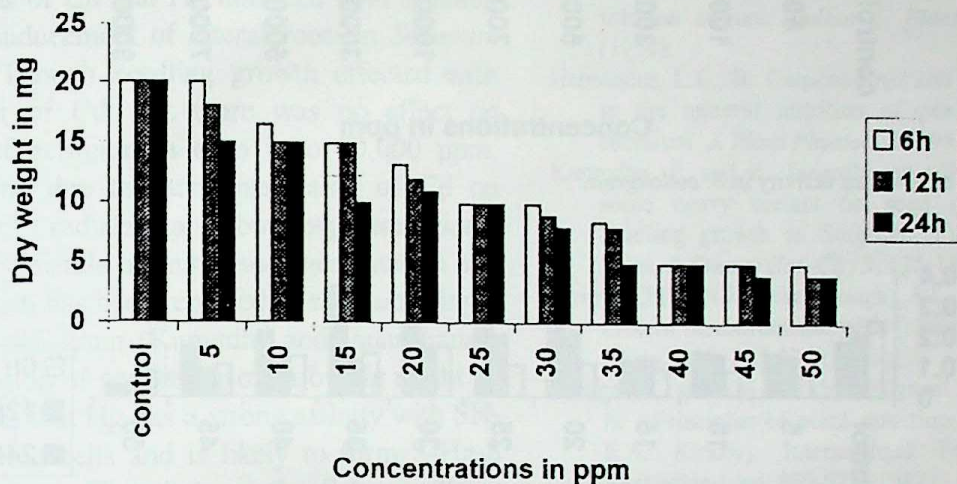


Fig. 4 : Effect of Hg on dry weight in *S. melongena*.

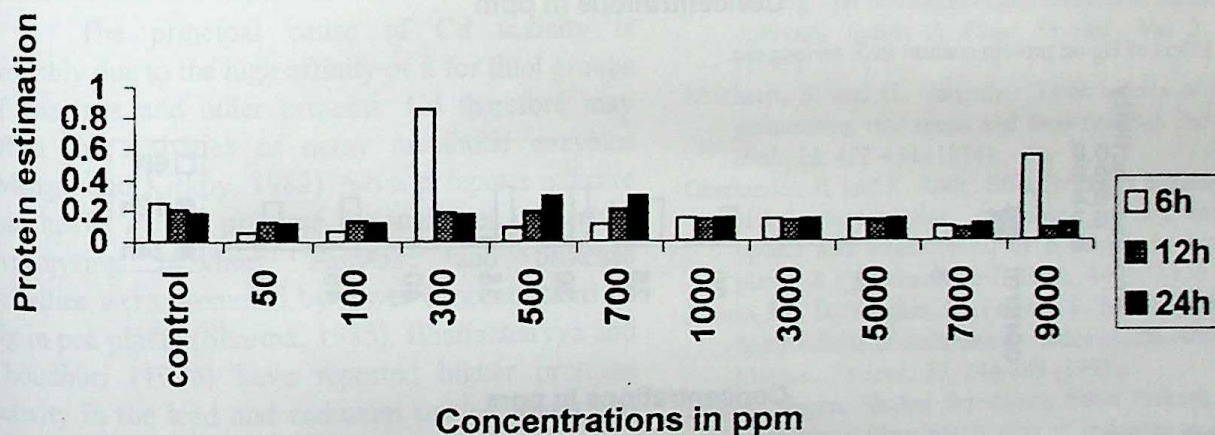


Fig. 5 : Effect of Cd on protein content in *S. melongena*.

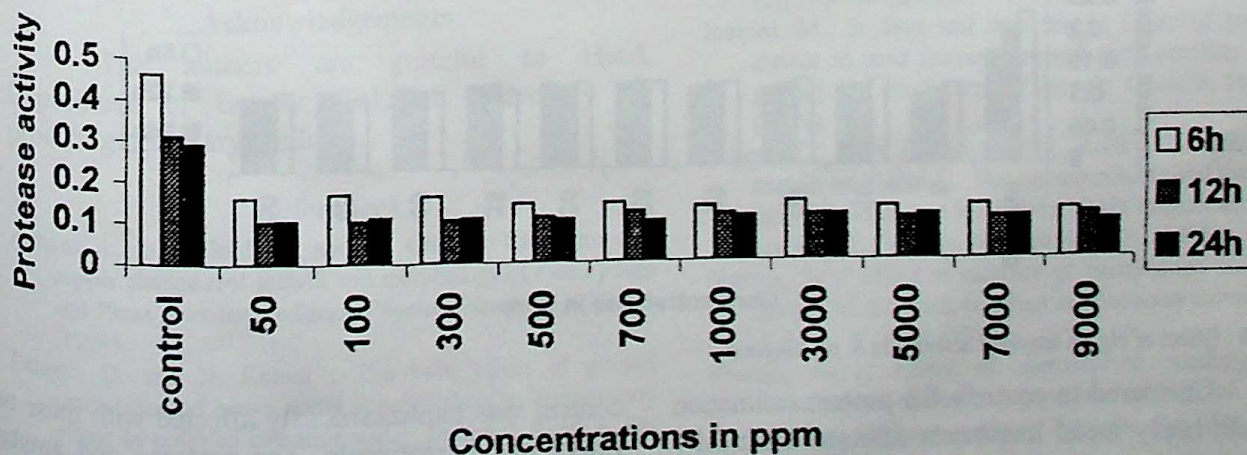
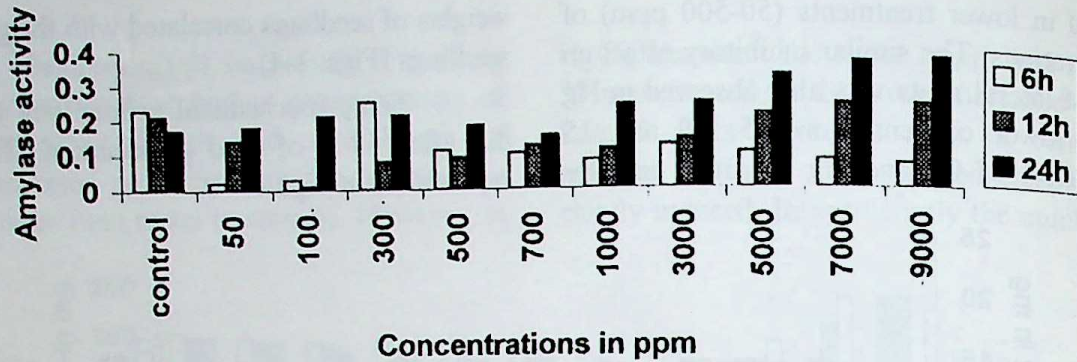
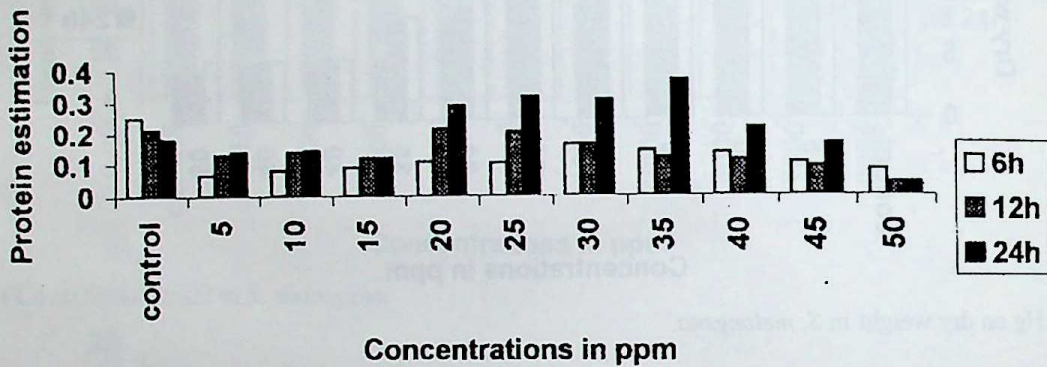
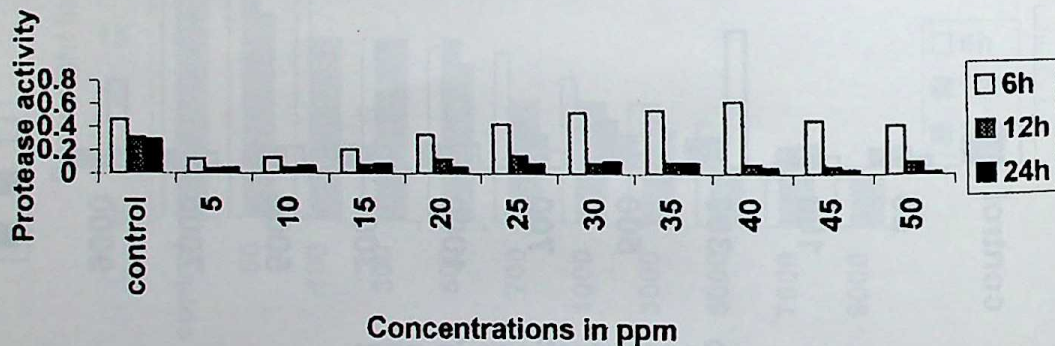
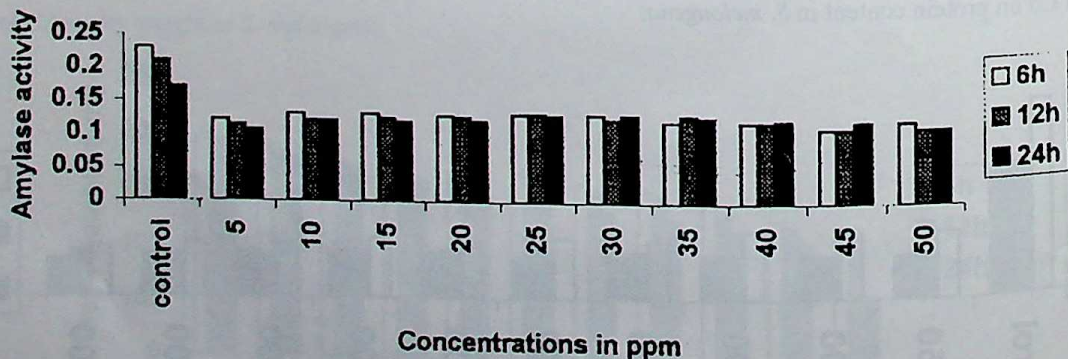


Fig. 6 : Effect of Cd on protease activity in *S. melongena*.

Fig. 7 : Effect of Cd on amylase activity in *S. melongena*.Fig. 8 : Effect of Hg on protein content in *S. melongena*.Fig. 9 : Effect of Hg on protease activity in *S. melongena*.Fig. 10 : Effect of Hg on amylase activity in *S. melongena*.

Compared to controls the protein estimation for both heavy metal treatments affected drastically in higher concentrations. However, the protein

content was haphazardously affected with these two heavy metal treatments. The protease and amylase activities were also suppressed in higher

concentrations compared to controls. Protease and amylase activities increased in some higher concentrations of Hg and Cd for 6 h and 24 h respectively.

In the present study, relatively higher concentrations of Cd and Hg inhibited both seedling growth and inducement of lateral roots in *Solanum melongena*. Though seedling growth effected with the treatment of Cd, but there was no effect on percentage of germination even upto 20,000 ppm. This might be due to inert interaction of Cd on emergence of radicle in laboratory conditions. Earlier, heavy metals inhibited seed germination and seedling growth has been reported in sorghum, finger millet and green gram (Kumudha and Janardhanan, 1986). Inhibition of seedling growth by Hg might be due to the fact that Hg has a strong affinity with SH-groups in living cells and is likely to form S-Hg-S bridges and thus affect the growth of the seedlings (Thimann and Bonner, 1949).

The principal cause of Cd toxicity is probably due to the high affinity of it for thiol groups of enzymes and other proteins. Cd therefore may affect the activities of many metabolic enzymes (Mengel and Kirkby, 1982). Several reports indicate that heavy metals promote the activities of certain hydrolysing enzymes. Amylase and protease activities were promoted by lower concentrations of Hg in pea plants (Sharma, 1985). Bhattacharyya and Choudhuri (1994) have reported higher protease activity in the lead and cadmium treated *Vigna* and *Hydrilla* plants.

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Heavy metal toxicity on dehydrogenase activity on rhizospheric soil of ectomycorrhizal pine seedlings in field condition

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Abstract : The present investigation was carried out to study the toxicity of heavy metals (Zn, Cd, Cu, Ni, Pb and Al) on the dehydrogenase activity of ectomycorrhizal (*Suillus leutus*, *Scleroderma aurantium*, *Cenococcum graniforme* and *Boletus* spp.) and non-mycorrhizal rhizospheric soil of pine seedlings. The treatment of heavy metals adversely affected the dehydrogenase activity. Inoculation of mixed ectomycorrhizal fungi harbored increased activity of dehydrogenase. It was observed that in absence of ectomycorrhizae, heavy metals drastically reduced the enzyme activity at higher concentration of metals.

Key words : Ectomycorrhizae, Heavy metals, *Pinus kesiya* seedlings.

Introduction

Mycorrhizal inoculation improves plant productivity especially in soils with low nutrient status. Non-mycorrhizal seedlings however, fail to survive natural planting sites, especially during a period of environmental stress (Anderson and Rygiewicz, 1991). Ectomycorrhizal fungi modify the morphology of roots and this change is correlated with the production of growth regulators, which increase the physiological process of the roots. The activity of any particular enzyme in soil is a composite of activities with various biotic and abiotic factors (Tewari *et al.*, 1987). Dehydrogenase activity in soil provides correlative information on the microbial population. Microbial biomass and their dehydrogenase activity have been observed influenced by rhizosphere of the host plant (Speir *et al.*, 1980). The use of heavy metals has increased due to fungicides, pesticides and industrial discharges. However, many fungi show remarkable ability to survive and grow in high concentrations of heavy metals. Toxic metals are numerous and vary in their action (Gadd, 1993). There are evidences that ectomycorrhizal fungi can evolve metal tolerance (Egerton-Warburton *et al.*, 1995). Successful tree establishment on contaminated sites has economic benefits (Denny and Wilkins, 1987). The survival of seedlings on contaminated sites determines its sensitivity to metal toxicity. The

accumulation of metals may be concentrated in the tissues. Mycorrhizal fungi may bind metals and thus detoxify them. However, studies on mycorrhizae have received enough attention during the past few years, however, their interaction in relation to environmental factors need careful investigation particularly in relation to soil pollution caused by heavy metals.

Materials and Methods

The study was conducted at the pine stand of permanent campus, North-Eastern Hill University, Shillong (altitude 1500 m, msl latitude 25° 34' N, longitude 91° 54' E) situated in the East Khasi Hills District, Meghalaya. Two sites were selected on a gentle slope and microplots were prepared and irrigated as needed. The seeds of *Pinus kesiya* were surfaced sterilized (5% NaOCl) and sown in each microplot. Seedlings of one month old were inoculated with mixed ectomycorrhizal fungi namely *Suillus leutus*, *Scleroderma aurantium*, *Cenococcum graniforme* and *Boletus* spp. The sporocarps of fungi were air dried and mixed with distilled water and inoculated (20 ml each) in each microplot. After confirming the mycorrhizal establishment, the seedlings were treated (10 ml each) with different concentrations such as Pb, Zn, Cd, Cu, Ni and Al. The control was not amended with heavy metals. The study was continued for one year.

Table - 1 : Correlation coefficient (r) values of mycorrhizal infection with dehydrogenase activity of rhizosphere soil of pine seedlings in field condition.

Parameter	Mycorrhizal infection	
	MMF	NM
Dehydrogenase	0.93*	NS

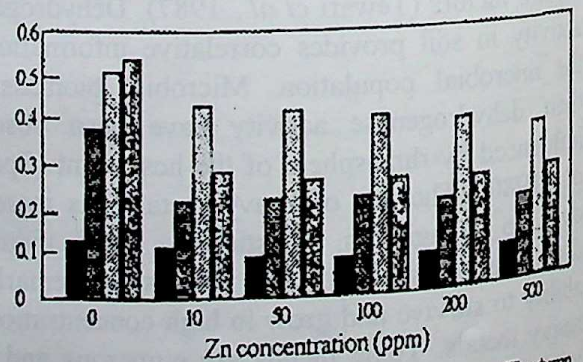
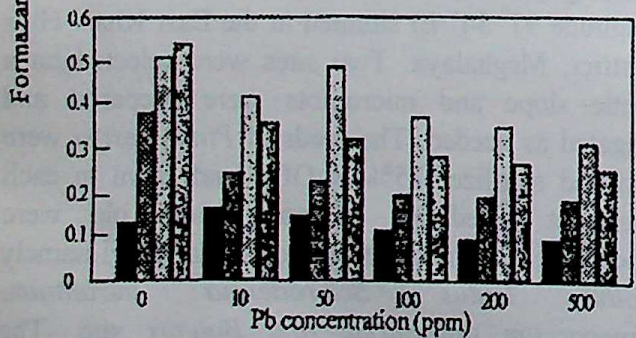
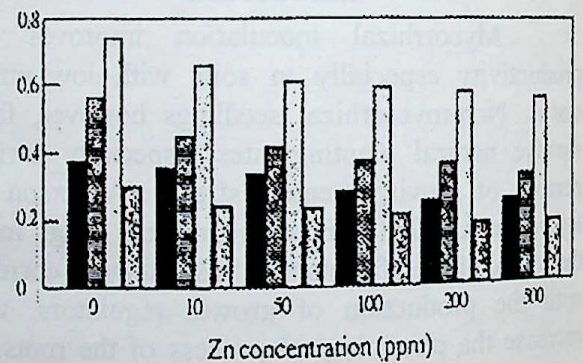
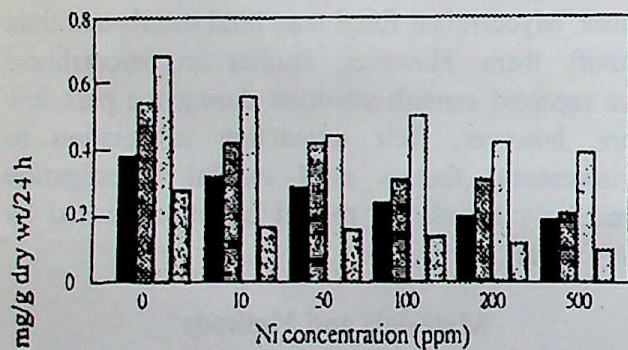
MMF= Mixed mycorrhizal fungi, NM= Non-mycorrhizal

*=Significant at $P>0.01$ level

Table - 2 : Analysis of variance (F) values with the sampling periods and mycorrhizal and non-mycorrhizal with various parameters in pine seedlings.

Source of variance	Variation between sampling periods	Variation between mycorrhizal and non-mycorrhizal
Dehydrogenase	6.71*	6.28**

* = Significant at $P>0.01$ level ** = Significant at $P>0.05$ level.

**Fig. 1 and 2 :** Dehydrogenase activity on rhizospheric soil inoculated with mixed mycobionts.**Fig. 3 and 4 :** Dehydrogenase activity on non-mycorrhizal rhizospheric soil.

2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) reduction technique was adopted for the evaluation of dehydrogenase activity in rhizosphere

soil (Casida, 1977). The data was processed by analysis of variance (ANOVA) and significant differences among means were identified with

Duncan's multiple range test at $P=0.01$ and 0.05 level. Correlation coefficient was also calculated.

Results and Discussion

Results showed that the enzyme activity was subjected to seasonal variation. Dehydrogenase activity was apparently less at the high concentrations of heavy metals. Addition of ectomycorrhizal fungi to seedlings increased the dehydrogenase activity (Figs. 1 and 2). Maximum inhibition (38%) was observed in Pb treated rhizosphere soil in contrast to Zn treated (25%) soil. In control, higher (46%) was observed in Zn and Pb treated ones (Figs. 3 and 4).

There was a negative correlation between heavy metals concentration and the activity of dehydrogenase. A positive correlation was found between mycorrhizal infection ($P>0.01$) and the activity of dehydrogenase (Table 1). There was a significant variation between sampling periods ($P>0.01$). A significant variation was also found between mycorrhizal ($P>0.05$) and non-mycorrhizal ones (Table 2).

The results suggest that the heavy metals have deleterious effect on activity of dehydrogenase. Probably the presence of ectomycorrhizal fungi enabled the seedlings to have increased enzyme activity. Jha et al. (1991) correlated the high concentration of dehydrogenase enzyme to high microbial population. Seasonal variations appear to be dependent on factors such as aeration, soil moisture, soil temperature and microflora (Burns, 1978). The results indicated that the degree of inhibition is dependent on metals added. This could be due to their toxic nature when present in high amount. The microorganisms in rhizosphere region may also differ in their sensitivity to metal toxicity. Reddy and Faza (1989) also reported decreased dehydrogenase activity in soil containing heavy metals. Greater inhibition of enzyme in the presence of heavy metals could be due to their inhibition of

enzyme sites. Stott et al. (1985) reported that metals like Cd, Zn, Ni, Cu and Pb might inhibit enzyme reaction by complexing the substrate. Tyler (1981) also observed that heavy metals inhibited a variety of microbial enzymes. Low inhibition of enzyme activity in mycorrhizal roots could be due to binding of metal ions by fungal mycelium, which consisted of mantle and Hartig net.

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Dental fluorosis in bovine of Nayagarh district of Orissa

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Abstract : Chronic fluoride toxicity in the form of dental fluorosis was observed in cattle from nine (9) villages under two (2) blocks of Nayagarh district of Orissa. Out of 1117 cattle, 221 (18.09%) showed the signs of dental fluorosis. In all affected villages, the prevalence of dental fluorosis in calves (< 1 year age) was greater than adults. There was significant difference in prevalence in respect to age. The commonly observed signs of dental fluorosis were brown discoloration, mottling, attrition or uneven wearing of teeth with or without pitting. None of the affected animals showed characteristic signs of osteofluorosis. The mean serum and urine fluoride concentration of affected animals were significantly ($P < 0.05$) higher than those of control animals. Fluoride levels (mean) of ground water and surface water in two blocks were 1.30 ± 0.16 ppm, 0.66 ± 0.08 ppm and 1.12 ± 0.19 ppm, 0.48 ± 0.05 ppm respectively. The fluoride content of grass samples of affected and control (non-endemic) area was comparable. There was a highly positive correlation ($r = +0.664$) between prevalence of dental fluorosis and fluoride content of ground water. It was concluded that fluoride intake through the water especially ground water contributed to the development of fluorosis in cattle.

Key words : Fluoride, Dental fluorosis, Cattle, Nayagarh, Orissa.

Introduction

Chronic fluoride toxicity or fluorosis is a world wide health problem in both human beings and animals. It is endemic in those areas where the fluoride content of drinking water is very high (Princi, 1960 : Stevenson and Watson, 1960). It is manifested mainly by two forms- dental fluorosis and osteofluorosis. In India, more than fifteen states have been declared as endemic for fluorosis by Rajeev Gandhi National Drinking Water Mission where large human population are affected with this chronic toxicological problem (Susheela, 1993). Although not much investigations appear to have been taken up to evaluate the impact of endemic fluorosis on livestock in many of those areas, however, literature revealed prevalence of hydrofluorosis in bovine in Gujarat (Kerur, 1971), Andhra Pradesh and Uttar Pradesh (Dwivedi *et al.*, 1997), Punjab (Sharma *et al.*, 1997) and Rajasthan (Choubisa, 1999). On the contrary, fluorosis in human beings has been reported from Nayagarh district of Orissa known to have high fluoride content in drinking water (Susheela, 1993 : Ray, 2000). But the literatures are absolutely silent about the status

of endemic fluorosis in animals which are sharing same environment. The present study was therefore undertaken to remedy this deficiency. It was aimed to study the prevalence of fluorosis in bovine as well as fluoride distribution in water and fodder and their interrelationship with prevalence.

Materials and Methods

A survey was carried out in twenty two (22) randomly selected villages of Odagaon and Bhapur blocks of Nayagarh district for the presence of fluorotic signs in ruminants particularly cattle (Fig. 1). A questionnaire was formed to record the information regarding, name of owner, species, age, sex of animals, period of inhabitation, type of feeding (stall fed/grazing) and sources of drinking water (tube well/Dug well/pond). To record the prevalence of fluorosis, house to house survey was made in the morning and evening when animals were available. Clinical examination was performed in each animal to note changes in general body condition, skin, muzzle, appetite, joints, bones and hooves. Special attention was paid for signs of (i) dental fluorosis viz.- mottling, staining, pitting, erosion, excessive

wearing and (ii) osteofluorosis viz.- lameness, stiffgait, bony exostoses, unthriftiness (Kolstad and Suttie, 1978 : Radostitis *et al*, 1994 : Choubisa *et al*, 1996). Presence of varied dental changes with or without other clinical signs was taken as criteria for fluorosis in animals. Blood samples were collected from jugular vein from 20 affected animals and serum was harvested after clotting of blood in plastic vials and placed in ice box. Then samples were transported to laboratory for fluoride analysis. Similarly urine samples collected from same animals in polyethylene bottle in the early morning were transported to laboratory for fluoride analysis in the ice box. Both blood and urine samples collected from 20 healthy cattle from Bhubaneswar, a non-endemic area for fluorosis served as control. Water samples from dugwell, tube well and pond from each endemic village were collected in polyethylene bottle during morning hours. Similarly water samples from different sources were also collected from Bhubaneswar. Serum, urine and water samples were stored in refrigerator for analysis of fluoride.

Representative forage samples (4) from each village were collected during morning hours. After proper cleaning and air drying these samples were then stored in separate plastic bags and brought to the laboratory for determination of the fluoride content. Four forage samples from control area were also collected. The forage samples were processed as per ISI method for fluoride analysis. The fluoride concentration in serum, urine, water and forage samples were measured in ppm by ion specific potentiometry using total ionic strength adjustment buffer (TISAB) following the method outlined in A.O.A.C. (1980) using expandable Ion Analyzer (Orion make, Model No. EA 940). Before analyzing the sample, instrument was calibrated with 1 ppm, 10 ppm and 100 ppm fluoride standard solutions. Statistical analysis was made using the standard method of Snedecor and Cochran (1967).

Results and Discussion

A total of nine (9) villages of Odagaon and Bhapur blocks under Nayagarh district were found

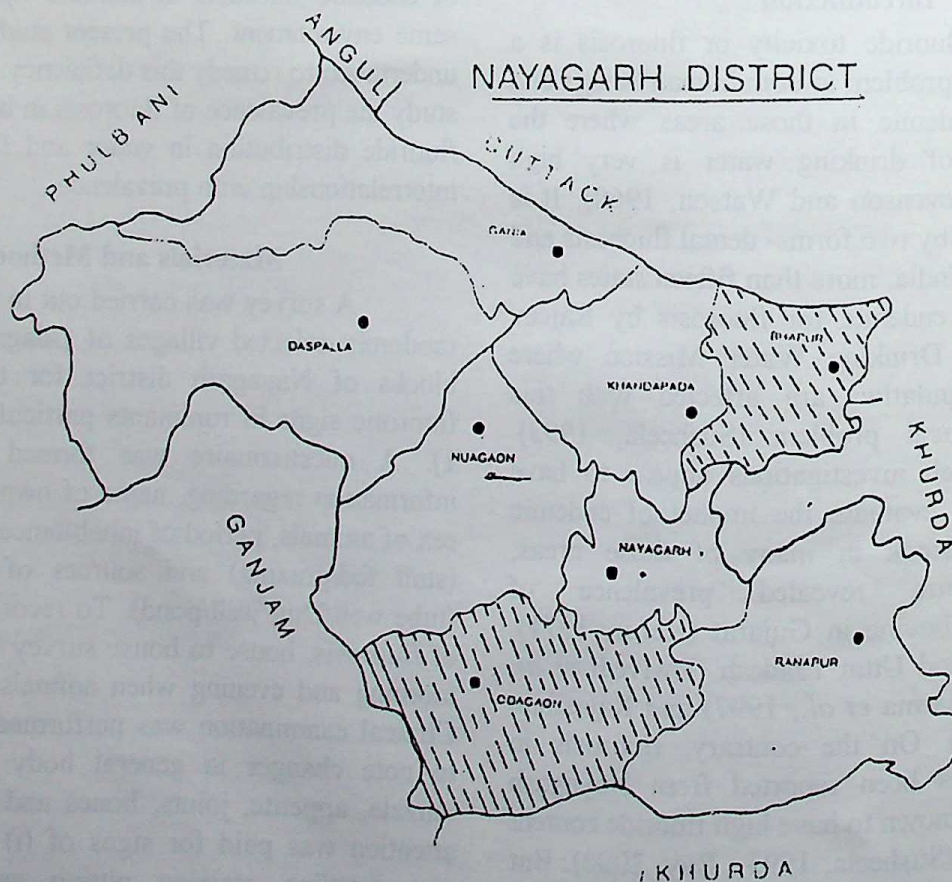


Fig. 1 : Map showing the study area of Nayagarh District.

to have fluorosis cases in animals. A total 124 (20.49%) of 605 cattle in Odagaon and 97(18.94%) of 512 cattle in Bhapur showed signs of dental fluorosis. The overall prevalence of dental fluorosis in cattle in nine affected villages was 18.09 per cent. The prevalence of dental fluorosis in different villages of two blocks has been shown in Table-1 and 2 respectively. The maximum prevalence of dental fluorosis was 30.43% where as minimum was 7.2%. In all study villages, the occurrence of fluorosis was highest in calves (< 1 year age) and lowest in adult cattle. Statistical analysis revealed significant difference in prevalence rate in respect to different age group of animals. But difference in prevalence was non significant ($P < 0.05$ by Chi-square test) in regard to sex.

Dental lesions such as brown discoloration, mottling, attrition or uneven wearing of teeth with or without pitting were most commonly recorded clinical signs of affected animals (Fig. 2, 3 and 4). The attrition of varied degree was noticed mostly in adult animals (Fig. 5). It was also noted that animals which were brought to affected area after eruption of teeth did not exhibit severe dental lesions. Choubisa

(1999) also recorded highest (100%) prevalence of dental fluorosis in calves (< 1 year age) than adult cattle. Shupe (1967) has stated that developing teeth are extremely sensitive to excessive fluoride. Once teeth have formed, calcified and erupted, fluorine ingestion will have little or no discernible effect on them. Radostitis *et al* (1994) also reported that deposition of fluoride in teeth occurs during or before eruption. In addition, calves ingest more fluoride through water and milk and so having a greater chance of exposure to fluoride. The above fact attributed to higher prevalence of dental fluorosis in calves (< 1 year) as well as variation in dental lesion in different age groups of cattle. Many affected animals showed general unthriftiness, rough skin, and low milk production which might be the result of excess fluoride ingestion. Earlier reports (Swarup and Singh, 1989 : Sharma *et al*, 1997 : Choubisa, 1999) also recorded similar non-specific clinical signs in fluorosis. However, osteofluorotic signs were not observed in any affected animal. Dwivedi *et al*. (1997) recorded lameness only in one, out of 29 animals affected with dental fluorosis in fluoride endemic villages of Nalgonda (U.P.).

Table - 1 : Age and sex wise prevalence of dental fluorosis in cattle in affected villages of Odagaon block in Nayagarh district.

Name of village		Below 1 year			1 - 3 years			Above 3 years			Grand total		
		Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
Dhologovindapur	No. examined	10	11	21	15	21	36	14	30	44	39	62	101
	No. affected	5	5	10	4	7	11	1	5	6	10	17	27
	% affected	50	45.45	47.61	26.66	33.33	30.55	7.14	16.66	13.63	25.64	27.41	26.73
Hanumantia	No. examined	13	27	40	13	11	24	40	65	105	66	103	169
	No. affected	6	12	18	5	4	9	3	11	14	14	27	41
	% affected	46.15	44.44	45	38.46	36.36	37.5	7.5	16.92	13.33	21.21	26.22	24.26
Madanpur	No. examined	21	30	51	20	30	50	48	75	123	89	135	224
	No. affected	9	13	22	5	9	14	3	9	12	17	31	48
	% affected	42.85	43.33	43.13	25	30	28	6.25	12.54	9.75	19.1	22.96	21.42
Baketara	No. examined	11	18	29	24	23	47	16	19	35	51	60	111
	No. affected	1	2	3	2	2	4	0	1	1	3	5	8
	% affected	9.09	11.11	10.34	8.33	8.69	8.51	0	5.26	2.85	5.88	8.33	7.2
Total	No. examined	55	86	141	72	85	157	118	189	307	245	360	605
	No. affected	21	32	53	16	22	38	7	26	33	44	80	124
	% affected	38.18	37.20	37.58	22.22	25.88	24.2	5.93	13.75	10.74	17.95	22.22	20.49

Table - 2 : Age and sex wise prevalence of dental fluorosis in cattle in affected villages of Bhapur block in Nayagarh district.

Name of village		Below 1 year			1 - 3 years			Above 3 years			Grand total		
		Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
Salapada	No. examined	12	27	39	14	21	35	20	25	45	46	73	119
	No. affected	4	10	14	3	5	8	2	4	6	9	19	28
	% affected	33.33	37.03	35.89	21.42	23.8	22.85	10	16	13.33	19.56	26.03	23.52
Khairagadia	No. examined	8	15	23	10	20	30	16	24	40	34	59	93
	No. affected	2	4	6	2	4	6	1	3	4	5	11	16
	% affected	25	26.66	26.08	20	20	20	6.25	12.5	10	14.7	18.64	17.2
Mahulia	No. examined	9	12	21	8	17	25	16	20	36	33	49	82
	No. affected	2	3	5	1	2	3	0	1	1	3	6	9
	% affected	22.22	25.00	23.8	12.5	11.76	12	0	5	2.7	9.09	12.24	10.97
Elapada	No. examined	10	17	27	10	19	29	15	32	47	35	68	103
	No. affected	2	2	4	1	2	3	0	2	2	3	6	9
	% affected	20	17.64	14.81	10	10.34	10.34	0	6.25	4.25	8.57	8.82	8.73
Chhelia	No. examined	11	14	25	18	20	38	20	32	52	49	66	115
	No. affected	6	8	14	6	7	13	2	6	8	14	21	35
	% affected	54.54	57.14	56	33.33	35	34.21	10	18.75	15.38	28.57	31.81	30.43
Total	No. examined	50	85	135	60	97	157	87	183	220	197	315	512
	No. affected	16	27	43	13	20	33	5	16	21	34	63	97
	% affected	32	31.76	31.85	21.66	20.61	21.01	5.74	12.03	9.54	17.25	20	18.94

**Fig. 2 :** Depicting brown pigmentation and mild wearing of teeth in calf (< 1 year).**Fig. 3 :** Showing brown discoloration and mottling of teeth in a calf (< 1 year).

Contrary to this, Choubisa (1999) recorded osteofluorotic signs in cattle where fluoride concentration of drinking water was much higher than the present study area.

The mean serum fluoride concentration of affected cattle in Odagaon and Bhapur blocks were 0.450 ± 0.078 ppm and 0.328 ± 0.030 ppm respectively whereas those of urinary fluoride were 3.680 ± 0.257 ppm and 2.373 ± 0.121 ppm respectively. These values were significantly

($P < 0.05$) higher than serum (0.055 ± 0.002 ppm) and urinary fluoride (0.629 ± 0.028 ppm) level of control animals, which indicated higher body burden of fluoride thereby confirmed fluorosis in animals. Dwivedi *et al.* (1997) also reported serum fluoride level of 0.30 ± 0.03 ppm and 0.57 ± 0.05 ppm in animals of fluoride endemic villages of Nalgonda (A.P.) and Unnao (U.P.) district but the urinary fluoride level was higher (8.46 ± 1.12 ppm and 10.64 ± 1.23 ppm respectively) than the present

study. The present findings corroborated the earlier observations of Hillman *et al.* (1979), who also reported that urinary fluoride level in fluorotic cows

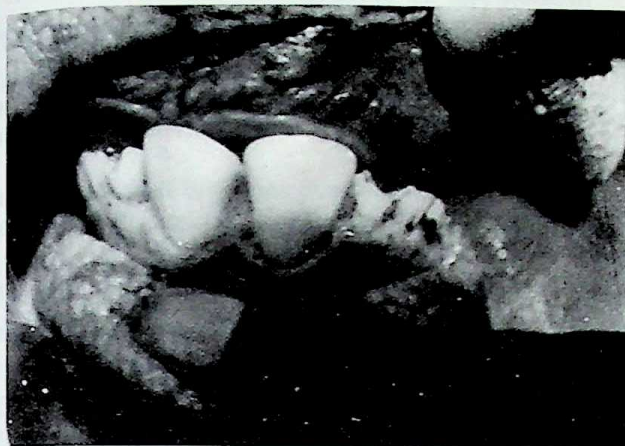


Fig. 4 : Demonstrating excessive wear, pitting and brown staining of teeth in a calf (> 1 year).

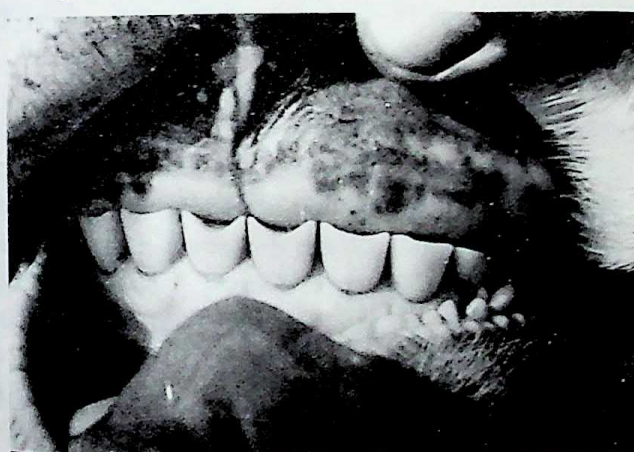


Fig. 5 : Showing excessive wearing of incisors giving a wavy appearance of Table surface in a bullock of 6 years.

varied from 1.04 to 15.7 ppm with an average of 5.13 ppm. In the present study, majority of the affected animals were young cattle where longer time is needed to reach equilibrium between skeletal and kidney in regard to fluoride. A major portion of fluoride ingested by young and growing animals is retained by mineralizing bone structure whereas amount of fluoride eliminated through urine is lowered.

Fluoride levels (mean) of ground water and surface water in Odagaon and Bhapur blocks were 1.30 ± 0.16 ppm, 0.66 ± 0.08 ppm and 1.12 ± 0.19 ppm, 0.48 ± 0.05 ppm respectively whereas in

Bhubaneswar the fluoride levels of ground water and surface water were 0.11 ± 0.04 ppm and 0.25 ppm respectively. The mean fluoride levels of grass in two blocks were 9.23 ± 0.09 ppm and 5.55 ± 0.58 ppm whereas that of control area was $4.5 \text{ ppm} \pm 1.5 \text{ ppm}$. In the present study fluoride concentration of ground water in affected area was much higher than that of control area whereas the fluoride level of surface water and forage samples of affected areas were comparable with that of control area. There was a highly significant positive correlation ($r = + 0.664$) between prevalence of dental fluorosis and fluoride content of ground water but correlation ($r = + 0.429$) was non-significant with surface water. The fluoride content of grass was lower than the tolerance limit of 40 ppm (Krook and Maylin, 1979). Thus, it was concluded that fluoride intake through the ground water surely contributed to the development of fluorosis in cattle.

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Diversity of ground arthropod community at organic and chemically intensive tea plantation of Darjeeling terai

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Abstract : Tea in Darjeeling foothills and terai are grown conventionally, with application of chemical fertilizers and pesticides, as well as organically without these inputs. Ground level arthropod community was collected from the above two types of tea plots using pitfall traps. Catches from these environments showed variation in the arthropod faunal structure with numerically and taxonomically greater abundance in the organic than that of the conventional plot. Coleopterans were more diverse with largest number of families and Recognizable Taxonomic Units (or morphospecies) in the organic tea plot. The diversity and similarity indices for coleopterans were comparable, in organic and conventional tea plots at species and family levels. The close relationship of the indices suggested that diversity study at family level could be used as surrogate for species level diversity; thus alleviating the laborious and expertise job of taxonomic identification of arthropod species. Faunal diversity study at ground level gave the clue that soil of the organic plantation was healthier than that of the conventional tea plot.

Key words : Arthropod community, Ground level, Tea plots, Faunal diversity.

Introduction

Tea being a perennial crop is grown in monocultures, interrupted only by shade trees. The monoculture provides a stable environment, food, and a breeding place for insects, mites and worms. Most of the dead plant tissues fall on the ground, making the surface and the sub-surface of soil an abode of great biological activity. A diverse array of soil arthropods is active in tea areas (Banerjee, 1993). Besides, these organisms also serve as pedobiological monitors, indicating the quality of habitat and/or its degradation (Luff *et al.*, 1989). The soils rich in biodiversity support healthy growth of plantation crops, and are termed as living soils, and form the basis for organic farming (Nampoothiri, 2001). To quantify the quality of habitats at ground level of the plantation, the study of the incidence and diversity of soil-surface arthropod fauna is important.

Pitfall traps are most commonly used for sampling ground-dwelling insects such as carabids and staphylinid beetles, spiders and predatory mites. The containers that can be used as pitfall traps are many and varied, but round plated pots and glass jars are the most popular (Jervis and Kidd, 1996). A liquid preservative is often placed in the trap to kill

and to preserve the catch, thereby reducing the risk of escape and predation within the trap, particularly of small individuals by the larger ones. Gist and Crossley (1973) have found that catches in pitfall traps might yield meaningful data and understanding of faunal diversity, provided proper attention is paid to potential sources of variation.

Since identification of a large variety of arthropods is very difficult, Gullan and Cranston (1994) have suggested that studies at community level in a particular area could yield more valuable information than any single species study. In order to alleviate taxonomic difficulties, they suggested selection of one or higher taxonomic groups instead of species level identification from amongst the organisms collected. As a second approach, Speight *et al.* (1999) recommended the use of morphospecies, 'recognizable taxonomic units' (RTUs) or 'operational taxonomic units' (OTUs) to overcome the taxonomic problem of determination.

The present study was an attempt to analyze taxonomically the pitfall trap catch upto the morphospecies level, and to determine the dynamics of incidence of the coleopterans on the ground, through six productive months of tea. The study further contemplated to compare the environmental

conditions occurring at the ground in organic and conventional tea-growing plots through careful analysis of the arthropod fauna caught in the pitfall traps.

The family is the most conspicuous and easily identifiable category. Each family usually presents a general facet that is recognizable at a glance and most of its entire species occupy a similar niche in their particular communities (Mayr and Ashlock, 1991). An attempt was also made to find whether higher taxonomic categories such as family diversity could be used as a substitute/surrogate for species level diversity, thus helping in circumventing the species identification problem by the ecologists or non-specialists.

Materials and Methods

Sampling was carried out at weekly intervals using pitfall traps, deployed in a randomized complete block design. A set of five traps was laid out in an organic tea plot as well as in a tea plot treated conventionally with mineral fertilizers and Tea Research Association approved pesticides (Anonymous, 1997). This deployment of the pitfall traps gave an opportunity for comparative study of the catches from the two differentially treated tea plots. The plots selected for the present study were located in a typical terai belt.

The traps used were made of glass, 11cm long and 6 cm in diameter with formaldehyde (4%) as a preservative (Vennila and Rajagopal, 2000). The traps were set in the soil with their brim flushed with the soil surface. The preservative added in each trap was one-third its volume. Polythene sheets cut into square shapes were used as covers to protect the traps, especially from rain.

Sampling was carried out during a period of six summer and monsoon months (April to September) with twenty-four weekly catches when the tea leaf yield in North-eastern region of India is the highest (Banerjee, 1993). The trapped insects were collected by straining the preservative. The traps were replaced in the same position. The collections from both the plots were then brought to the laboratory and sorted taxonomically. Coleopterans, which were recorded as the major

catch, were analysed upto 'recognizable taxonomic unit' (RTU) or 'operational taxonomic unit' (OTU) levels, deemed equivalent to morphospecies. According to the concept of RTU/OTU, the classification of the specimens was done by considering their morphological features, which were then grouped to comprise morphospecies. Oliver and Beattie (1993, 1996 a, b) has described this method in detail and tested with case studies. Diversity (Shannon and Weaver, 1963) and similarity (Sorensen, 1948) indices were calculated at morphospecies and family levels, in both the habitats.

Results and Discussion

An overall analysis of the arthropod fauna collected in the pitfall traps in organic tea plot showed a greater abundance of the representatives of Coleoptera, Hymenoptera, Lepidoptera, Acari, Collembola and terrestrial crustacean order, Isopoda, along with the orders Neuroptera and Scolopendromorpha that constituted the exclusive predatory groups. The conventional tea plot catch in general indicated a fair number of orthopterans, dermapterans, isopterans, hemipterans, dictyopterans, diplopodans, and spiders. Greater occurrence of the major players of the detritus food chain such as isopodan crustaceans, springtails (collembolan), diplopodans, and a large number of coleopteran species was recorded in the organic tea plot. It was clearly evident that the coleopterans were consistently more abundant in the organic tea plot than in the conventional one, although there were periodic surges in population abundance in both the plots (Fig. 1). Detailed analysis of coleopteran families and morphospecies of six samples spread through five months of the study clearly indicated that the number of individuals and families were, in general, greater in organic tea plots compared to the conventional ones (Table-1). Further, the increase in the number of morphospecies or RTU of coleopterans had a corresponding increase in the number of its families. The positive relationship between these two variables was evident from the trend line diagrams, both for the samples taken in organic and in the conventional tea plots (Fig. 2).

Diversity of ground arthropods of tea plantation.

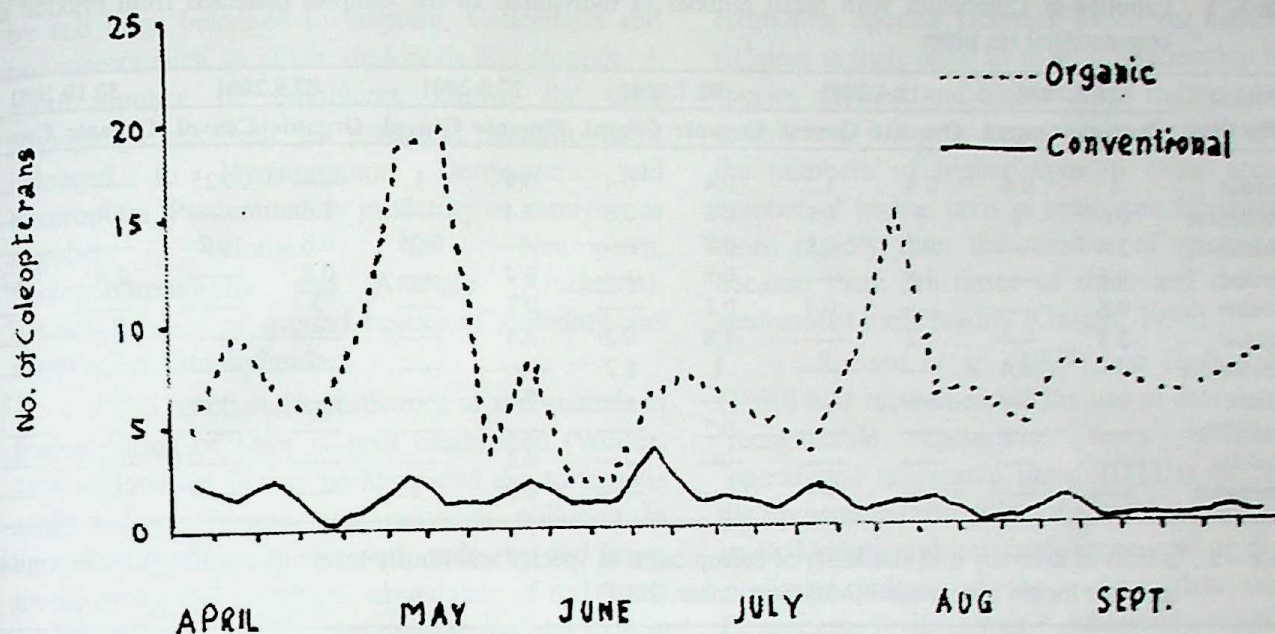


Fig. 1 : Weekly occurrence of coleopterans in organic and conventional tea plots.

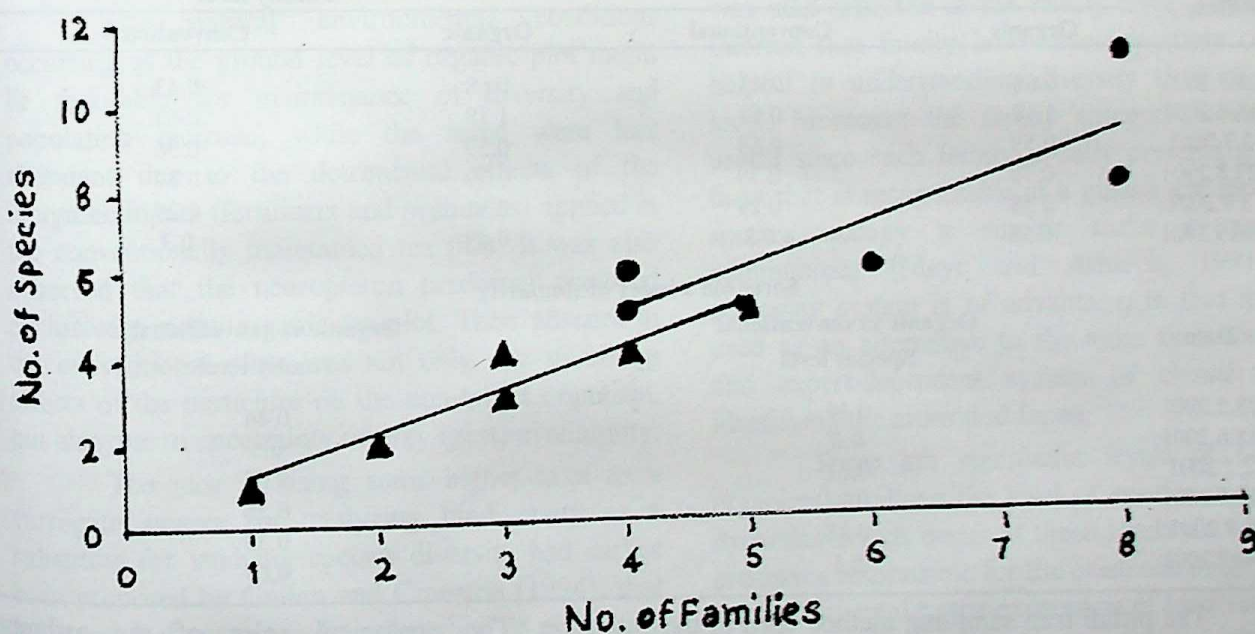


Fig. 2 : Relationship of coleopteran families and species occurring at organic (●) and conventional (▲) tea plots as observed from six pitfall trap catches (April to September, 2001).

With the application of Shannon's General Diversity Index, it was evident that diversity at the morphospecies (RTUs) level was almost similar to the diversity at the family level (Table-2). Further, similarity index values were also found closely comparable at both the taxonomic categories, based on the samples collected from the two different habitats (organic and conventional).

Soil supports a vast diversity of organisms. From the ecological point of view, the functional significance of soil biota are those associated with organic matter decomposition, mineralization of nutrients and synthesis of humic compounds. The synergistic relationship of soil animals with free living and symbiotic microorganisms is responsible for rapid cycling of essential nutrients (Lee, 1991).

Table - 1 : Families of Coleoptera with mean number of individuals in the samples collected from organic and conventional tea plots.

Date of collection	28.5.2001		18.6.2001		02.7.2001		27.8.2001		03.9.2001		30.10.2001	
	Organic	Convnl.	Organic	Convnl.	Organic	Convnl.	Organic	Convnl.	Organic	Convnl.	Organic	Convnl.
Histeridae	1	0.4	0.4	1.2	0.4	0.4	0.4	1	-----	0.25	-----	-----
Staphylinidae	0.6	1	3	1.6	0.8	0.6	2.4	-----	1.6	-----	1.4	-----
Psephenidae	2.4	-----	0.2	0.2	0.8	-----	-----	0.25	0.6	0.2	-----	-----
Elateridae	0.6	-----	1.8	-----	0.8	-----	0.4	-----	0.8	-----	2.6	0.2
Carabidae	0.6	-----	-----	0.4	0.2	-----	0.2	-----	3	-----	-----	-----
Cucujidae	2.8	-----	1.2	-----	1.8	0.2	3.4	-----	-----	-----	4.2	-----
Tenebrionidae	-----	0.4	-----	-----	1	0.2	-----	-----	-----	-----	-----	-----
Silphidae	-----	-----	-----	0.8	-----	-----	0.6	-----	-----	-----	0.4	0.2
Coccinellidae	-----	-----	-----	-----	0.2	-----	-----	-----	-----	-----	-----	-----
Scaradidae	-----	-----	-----	-----	-----	-----	0.2	-----	-----	-----	-----	-----
Dermestidae	-----	-----	-----	-----	-----	-----	0.2	0.25	-----	-----	-----	-----

Table - 2 : Indices of diversity and similarity of coleopterans at species and family levels in organic and conventional tea plots for six observation (May-September, 2001).

Shannon's general diversity index				
Date	Species level		Family level	
	Organic	Conventional	Organic	Conventional
28.5.2001	0.68	0.43	0.68	0.43
18.6.2001	1.18	0.61	1.18	0.61
2.7.2001	0.59	0.59	0.59	0.56
27.8.2001	0.79	0.39	0.79	0.39
3.9.2001	0.58	0.29	0.51	0.29
30.9.2001	0.58	0.3	0.49	0.3

Sorenson's index of similarity		
Date	Organic vs conventional	Organic vs conventional
	Species level	Family level
28.5.2001	0.44	0.44
18.6.2001	0.6	0.6
2.7.2001	0.67	0.67
27.8.2001	0.27	0.36
3.9.2001	0.28	0.33
30.9.2001	0.5	0.67

The pitfall trap sampling method used in the present study was found to be a useful tool in assessing biodiversity and the population changes of different guilds of ground-dwelling arthropod fauna inhabiting the tea-growing plots. Such a method has also been found to be very helpful in knowing the occurrence of certain species, in providing an idea of the species composition of a particular locality, and in classifying different habitats and their health, based on the arthropod communities (Eyre and Luff, 1990; Eyre *et al.*, 1990).

The ecological roles of the arthropods trapped require an understanding of their collective contribution to the ground-level ecology in two different situations, organic and conventional tea plots. Coleopterans predominates the arthropod community at ground level either as predators or as scavengers of decaying animals and/or vegetable matter (Raw, 1967). The members of other arthropod orders collected in the pitfall traps could broadly be classified under four feeding guilds. Herbivores were mainly from Orthoptera,

Homoptera, Lepidoptera and Diptera. Saprophages by and large belonged to Isoptera, Collembola and non-insects such as mites, diplopods and isopods. A good number of omnivores known for their phytophagic as well as predatory/parasitic habit belonged to Hymenoptera, Dermaptera and Heteroptera. Predominantly predatory or carnivorous members belonged to Neuroptera, Scolopendromorpha and Araneae (Arachnida), besides families of ground beetles (Carabidae) and rove beetles (Staphylinidae).

The functional significance of soil animals in fragmentation of litter is well established (Weiser, 1984). However, it may be hampered due to various anthropogenic factors and pesticide pollution in agricultural soil. In the present study, by and large, the diversity and numerical abundance of soil-fauna was found to be more in the organic tea plot than in the conventionally maintained one.

The natural environmental conditions occurring at the ground level of organic plot might be favorable for maintenance of diversity and population increase, while the same were less abundant due to the detrimental effects of the inorganic inputs (fertilizers and pesticides) applied in the conventionally maintained tea plot. It was also observed that the neuropteran predators occurred exclusively in the organic tea plot. Their absence in the conventional plots was not only due to killing effects of the pesticides on the non-target organism, but also due to uncertainty of prey (pest) availability.

The idea of using some higher taxa as a surrogate system for analyzing biodiversity as a substitute for studying species diversity had earlier been proposed by Gullan and Cranston (1994); that indicated that surrogacy-based method of estimating species richness was founded in the relationship between the numbers of species and the numbers of supra-specific taxa, such as family. Positive relationships between the number of species and higher taxa in different areas have been documented for a variety of extant groups (Williams and Gaston, 1994), which have also been found to be true in the present study of coleopteran fauna, as was evident from the analysis of the pitfall-trap catch.

The principal advantage of an approach of estimating species richness based on higher taxon richness is that, once an overall relationship between species richness and higher taxon richness has been established, it is only necessary to directly determine the numbers of higher taxa in other areas. The number of higher taxa in areas can be documented more rapidly than the number of species simply because there are fewer of them and they can be enumerated more readily (Gaston, 1996).

Speight *et al.* (1999) and Gadagkar *et al.* (1990) had recommended the use of morphospecies, 'recognizable taxonomic units' (RTUs) or 'operational taxonomic units' (OTUs) to overcome the taxonomic difficulties of identification at species level. Using graphical analysis as well as diversity/similarity indices of the present data on order Coleoptera, it was observed that the biodiversity/similarity at the morphospecies level was also reflected at the family level. Thus, it was inferred that family level diversity study could be helpful in understanding diversity even at species level. Moreover the family category is especially useful since each family usually presents a general facet that is recognizable at a glance and most of its species occupy a similar niche in particular communities (Mayr and Ashlock, 1991). The surrogate system is of advantage, in that it can be used as an alternative to the more time consuming and expert-dependent system of classifying and identifying the arthropod fauna.

There are significant levels of biological organization above the level of species and that the dynamics which occur at these levels as well as the processes responsible for the existence of these levels are fundamental to the expression of biodiversity. An ecological community is clearly a higher level organization, and the mechanics responsible for production of these levels are variously capable of influencing it if not regulating the biodiversity (Drake *et al.*, 1996).

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Studies on *Merops orientalis* Latham 1801 with special reference to its population in Mayiladuthurai, Tamil Nadu

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Abstract : Role of habitat-structure and climatic factors in the population dynamics of the small green bee-eater *Merops orientalis* was evaluated in three habitats viz., agricultural lands, river banks and human habitations during 1991-1993. The river banks supported relatively high population of bee-eaters ($157/\text{Km}^2$) followed by the agricultural lands ($101/\text{Km}^2$) and human habitations ($58/\text{Km}^2$). Bee-eater populations showed year-wise variations in river banks and human habitations having high values during 1992 ($123/\text{Km}^2$) and 1993 ($43/\text{Km}^2$) respectively. Agricultural lands showed a significantly low mean density in 1991 than other years. Seasonal variations in the bee-eater densities among the habitats were also recorded. Vegetation structure, food (insects) availability, climatic conditions and human disturbance were the casual factors for variations in bee-eater populations.

Key words : Population fluctuation, River banks, Agricultural lands, Human habitations, Food availability, Human disturbance.

Introduction

The small green bee-eater *Merops orientalis* plays a significant role in controlling agricultural insect pests in South India (Mathew *et al.*, 1978; Daniels, 1991). In spite of its undoubted significance in agro-ecosystems, reports on its ecology are few and sporadic (Ali, 1979; Ali and Ripley, 1983; Joshua and John Sings, 1988; Sridhar and Karanth, 1993), although extensive reports on its counterparts are available (Fry, 1984; Lessells and Krebs, 1989). A general concept is that birds select habitats based on vegetation structure or habitat physiognomy has been expressed by several researchers (Lack, 1933; Svardson, 1993; Odum, 1950; Hilden, 1965; James, 1971). Avian population densities can also be influenced by singular or interactive influence of predation, intra and interspecific resources, competition, parasites and diseases, habitat availability, and weather (den Boer and Gradwell, 1970; Andrewartha and Birch, 1954; Begon and Mortimer, 1986). The magnitude of the influence of these factors may vary in importance according to geographical area, food habits and migratory status of the birds (Lack, 1966; von Haartman, 1971; Newton, 1980; van Balen, 1980). The present paper deals with the factors that influence the population

fluctuations of the small green bee-eater *M. orientalis* in an agro-ecosystem in South India to evaluate the possible role in agricultural insect- pest management.

Materials and Methods

Study habitats : In the study area, three habitat types viz., 'river banks', 'human habitations' and 'agricultural lands' were demarcated and in each of them a, 2000 X 100m plot was marked for intensive bird censuses. River bank was characterized by the predominance of riverine vegetation, the 'human habitation' included areas with a predominance of human dwellings and the 'agricultural land' were under cultivations of paddy, sugarcane and plantain.

A one time survey on the vegetation types/ microhabitat availability in the three habitats was carried out during January, 1993 which yielded the results as shown in the Table given on page 478.

Population studies : Populations of the bee-eater were monitored in the three habitats from 1991 to 1993 by the line transect method described by Gaston (1975). Though many methods of avian population enumeration are available, the line transect method was adopted in the present study

Habitat	Microhabitats (%)				
	AC	GR	HD	WB	FL
RB	45.25	20.75	-	3.50	30.50
HH	16.99	26.23	42.35	3.63	10.80
AL	92.00	2.50	0.50	3.50	1.50

RB = River banks, HH = Human habitations, AL = Agricultural lands, AC = Agricultural crops, GR = Groves, HD = Human dwellings, WB = Water bodies, FL = Fallow lands.

because of the nature of study area which was predominantly open and almost uniform with sparse distribution of trees and hence detection of birds was not a problem and the probability of letting any birds unnoticed was very low.

At each study area, one 2 Km long transect was laid and the bee-eaters were censused by counting all the birds seen within a 50 m belt on either side of the transect. The data so obtained was extrapolated to estimate the bird densities as number Km^{-2} using the following formula :

$$\text{Density} = \frac{N}{2 \text{ LW}}$$

Where, N = Number of birds counted, L = Length of the transect and W = Width of the transect in kilometers

All census operations were carried out immediately after sunrise, and normally from 06.00 to 08.00 hrs. Double counting was avoided by noting the direction of movements of the birds. The census was done at the rate of 0.75 to 1.00 Km/hr. counting of birds was avoided on cloudy, rainy and windy days. Censuses were carried out fortnightly from January, 1991 to December, 1993 in all the three habitats. Monthly density values were calculated from the fortnightly census. Prey species (insects) were collected using standard sweep nets.

Results and Discussion

Densities of the bee-eater, *M. orientalis* in river banks, human habitations and agricultural lands from 1991 to 1993 are given in Table 1. The bee-eater population density varied between $25/\text{Km}^{-2}$ (the pre-monsoon of 1992 at the human habitations) and $157/\text{Km}^{-2}$ (summer of 1992 at the river banks). In general the river banks supported relatively higher numbers followed by the agricultural lands and human habitations. Within the habitats, the bee-eater

densities were highest during the summer of 1991 and 1992 and post-monsoon 1993 in river banks, and during winter in all the three years in human habitations. In agricultural lands the highest densities of bee-eaters were recorded during the pre-monsoon in 1991 and 1993, and during summer in 1992.

Mean densities (across all seasons) in the three habitats are given in Table 2 year wise. River banks and human habitations had higher mean densities during 1992 ($123 / \text{Km}^{-2}$) and 1993 ($43 / \text{Km}^{-2}$) respectively. However, the year wise difference was not statistically significant ($P > 0.05$) (Table 2). The agricultural lands had a significantly lower mean density in 1991 than during other years (ANOVA and SNK test) (Table 2).

Seasonal variations in the bee-eater densities are given in Table 3. Significant seasonal variations in bee-eater densities existed in the river banks and agricultural lands but not in human habitations (ANOVA) ($P < 0.05$). The river banks and agricultural lands had significantly lower densities during monsoon than in other seasons (ANOVA and SNK test). It is interesting to note that the bee-eater density showed a declining trend in the pre-monsoon seasons in human habitations whereas they showed an increasing trend during the same time in the other two habitats (Table 3).

Yearly variations in the relative availability of food indicated highest availability of insects in the agricultural lands and lowest in the human habitations. Only coleopterans had a significant year wise difference ($P < 0.05$) (Table 4).

Bee-eater densities showed habitatwise variations with the river banks generally supporting the highest populations and the human habitations the least. According to Lack (1933, 1937) and Hilden (1965), terrestrial animals seek their habitats rather than dispersing randomly and birds are no exception.

Table - 1 : Seasonal variations in the density (Km^{-2}) of the small green bee-eater, *Merops orientalis* in different habitats from, 1991-1993. Values are $X \pm \text{ISD}$ ($n=3$).

Year	Season	Habitats		
		RB	HH	AL
1991	Post-monsoon	110 ± 17	38 ± 20	66 ± 01
	Summer	135 ± 18	35 ± 09	72 ± 15
	Pre-monsoon	128 ± 08	28 ± 03	73 ± 15
	Monsoon	83 ± 03	38 ± 08	54 ± 03
1992	Post-monsoon	115 ± 05	42 ± 28	87 ± 13
	Summer	157 ± 10	38 ± 08	99 ± 12
	Pre-monsoon	140 ± 09	25 ± 15	85 ± 15
	Monsoon	78 ± 06	45 ± 05	70 ± 04
1993	Post-monsoon	132 ± 03	52 ± 08	91 ± 01
	Summer	118 ± 21	25 ± 05	98 ± 13
	Pre-monsoon	123 ± 13	38 ± 03	101 ± 18
	Monsoon	68 ± 10	58 ± 19	54 ± 09

RB = River banks, HH = Human habitations, AL = Agricultural lands.

Table - 2 : Yearly variations in the density (Km^{-2}) of bee-eater in the three habitats from, 1991-1993. Values are $X \pm \text{ISD}$ ($n=12$). Similar means are indicated by continuous horizontal lines under them (SNK - test).

Habitat	Year			ANOVA					
	1991	1992	1993	Source	SS	Df	MS	F	P
RB	114 ± 23	123 ± 32	110 ± 28	Between Years	934.72	2	467.36	0.601	0.554
				Among Years	25654.12	33	777.39		
HH	35 ± 11	38 ± 16	43 ± 20	Between Years	438.89	2	219.44	0.863	0.431
				Among Years	8391.67	33	254.29		
AL	66 ± 12	85 ± 15	86 ± 22	Between Years	2982.39	2	1491.19	5.191	0.011
				Among Years	9479.25	33	287.25		

RB = River Banks, HH = Human habitations, AL = Agricultural lands.

Table - 3 : Seasonal variations in the density (Km^{-2}) of bee-eater in different habitats from, 1991-1993. Values are $X \pm \text{ISD}$ ($n=9$). Similar means are indicated by continuous horizontal lines under them (SNK - test).

	Year				ANOVA					
	Mon.	Pom.	Prm.	Sum.	Source	SS	Df	MS	F	P
RB	77 ± 9	119 ± 13	131 ± 22	136 ± 22	Between Seasons	19538.89	3	6512.96	29.56	0.000
	POM	Sum.	Prm.	Mon.	Among Seasons	7050.00	32	220.31		
HH	44 ± 23	33 ± 9	31 ± 10	47 ± 14	Between Seasons	1083.33	3	602.77	2.749	0.059
	Mon.	Pom.	Prm.	Sum.	Among Seasons	7022.22	32	219.44		
AL	59 ± 9	81 ± 13	86 ± 18	90 ± 18	Between Seasons	5163.64	3	1721.21	7.547	0.001
					Among Seasons	7298.00	32	228.06		

RB = River banks, HH = Human habitations and AL = Agricultural lands.

Table - 4 : Yearly variations in the relative availability of insect (prey) categories in three habitat types from, 1992 to, 1993. Values are mean \pm 1SD (n=12). Differences between means were assessed by Students' 't' test.

Prey item	River banks				Human habitations				Agricultural lands			
	1992	1993	t	p	1992	1993	t	p	1992	1993	t	p
ORT	4.8 \pm 1.8	5.9 \pm 3.8	0.629	0.536	2.6 \pm 1.0	3.0 \pm 1.5	0.237	0.815	9.5 \pm 4.4	9.8 \pm 1.6	0.522	0.607
HEM	5.0 \pm 1.3	5.6 \pm 2.4	0.636	0.531	2.2 \pm 2.9	2.6 \pm 1.0	1.058	0.301	9.0 \pm 5.8	5.5 \pm 1.9	1.896	0.071
COL	4.9 \pm 1.7	4.8 \pm 3.0	0.559	0.582	2.9 \pm 1.5	3.3 \pm 1.5	0.730	0.473	6.8 \pm 3.7	10.3 \pm 2.7	2.796	0.011*
LEP	4.4 \pm 1.7	3.6 \pm 1.9	1.330	0.197	2.1 \pm 0.9	2.6 \pm 1.7	0.343	0.735	5.9 \pm 3.0	5.9 \pm 2.1	0.129	0.899
HYM	4.9 \pm 1.8	3.6 \pm 1.7	1.989	0.059	2.2 \pm 1.0	2.3 \pm 1.5	0.457	0.652	4.9 \pm 1.6	4.8 \pm 1.4	0.637	0.531
ODO	3.8 \pm 1.3	3.1 \pm 1.3	1.273	0.216	2.4 \pm 0.9	2.7 \pm 1.4	0.188	0.852	4.6 \pm 1.5	5.5 \pm 1.1	1.844	0.079
DIP	3.7 \pm 1.1	2.8 \pm 1.9	1.687	0.106	2.4 \pm 1.2	1.8 \pm 0.7	1.274	0.216	3.2 \pm 1.3	3.7 \pm 1.3	1.061	0.300
OTH	3.2 \pm 1.3	2.5 \pm 1.3	1.490	0.150	1.9 \pm 0.7	1.4 \pm 0.6	1.920	0.068	2.6 \pm 0.8	3.3 \pm 0.9	1.857	0.077
ALL	34.8 \pm 4.9	31.8 \pm 5.8	1.357	0.189	18.7 \pm 4.7	19.7 \pm 2.9	0.689	0.498	46.8 \pm 5.9	48.5 \pm 5.6	0.846	0.047*

ORT = Orthoptera, HEM = Hemiptera, COL = Coleoptera, LE = Lepidoptera, HYM = Hymenoptera, ODO = Odonata, DIP = Diptera, OTH = Others. * : Pairs of means significantly different at 5% level.

The greater number of bee-eaters at the river banks might be due to favorable features such as greater vegetation densities and suitable soils for nest excavation. Influence of habitat structure (characteristics) on the avian distributions had been emphasized by Anderson (1976). DeGraaf and Wentworth (1986) also reported a strong association between the measures of tree cover and insectivorous bird densities. Gole (1987) after studying the distribution of *M. orientalis* at Western Ghats, south of Bombay, reported a preference for thorny scrubs. The study area (river banks) resembled a scrub and bush type with short and stumpy vegetation. The high number of *Cassia occidentalis* at the river banks, which are used for perching, is another attractant to the bee-eaters. Availability of suitable sites for easy excavation of nests at the river banks is another contributing factor to their preference by the bee-eaters. Relationships between availability of nest sites and bird numbers had been well documented (Farner and King, 1971; Alatalo *et al.*, 1984; Cody, 1985). Cody (1980) stated that birds adapt to areas with suitable habitat, which provides nesting site, nesting material, food and protection from other species. The agricultural lands ranked second to river banks in bee-eater densities probably due to relatively rich supply of insects as pointed out by Lack (1966) that food is frequently the most important density dependent factor for birds. The least preference for human habitations, in general, might be due to lesser food availability coupled with

greater human disturbance. However, there is a preference for human habitations during winter months as the numbers of bee-eater increased markedly during that time. Role of weather, especially temperature and rainfall, in avian distribution and movement pattern, has been brought out by Andrewartha and Birch (1954). Moss *et al.* (1982) opined that weather can be important, either by itself or through its effect on food supplies. Animals seem to limit their own numbers below any threshold set by weather, food, disease, predation, parasites or places to live. From the foregoing discussion, it is apparent that the factors influencing the bee-eaters are vegetation, nest availability and food availability. A complete understanding of their habitat preference requires quantified information on births, deaths, immigration, emigration, intra and inter-specific interaction and predation. Williamson (1974) regarded four factors, which changed the size of a population and they are births, deaths, immigrants and emigrants. According to Welty (1982), intra and interspecific factors and predation are significant for understanding observed pattern of habitat selection. Since the role of above factors on bee-eater distribution could not be quantified, further research encompassing the above factors will throw more light on this aspect of bee-eater ecology.

Seasonal variations in the density of bee-eaters was recorded among habitats with river banks showing high densities during summer, the agricultural lands during pre-monsoon, and the

human habitation during monsoon season. The bee-eater breeds during summer (April-June) in the study area and since the river banks provided suitable soil conditions for nest excavation; they had a high number of bee-eaters during the summer. Relatively high densities of bee-eaters during pre-monsoon in agricultural lands might be attributed to the movement of newly recruited young ones together with their parents in pursuit of insect food, which was usually abundant in the agricultural lands. Human habitations had high population of bee-eaters during monsoon which suggests that the human habitation offered safer shelter for the bee-eaters against vagaries of monsoon weather which is characterized by high wind speeds and torrential down pour.

Various factors may be cited for yearly variations in bird densities viz., predation, intra and interspecific resource competition, parasites and diseases, habitat availability and weather (den Boer and Gradwell, 1970; Andrewartha and Birch, 1984; Begon and Mortimer, 1986) as also food habits and migratory status (Lack, 1966; von Haartman, 1971; Newton, 1980; van Balen, 1980).

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Comparative account of certain enzymes in the serum of homo-iothermal vertebrates subjected to production of myocardial infarction by isoproterenol hydrochloride

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Abstract : Myocardial infarction was produced by subcutaneous administration of isoproterenol hydrochloride (85 mg/kg b.w. for two consecutive days). The myocardial damage was proved by observing increase in the activity of SGOT and SGPT in serum whereas AChE activity was inhibited by increasing K_m , without affecting V_{max} . The inhibition of AChE and inhibitory kinetic may be useful in the diagnosis and management of salvage of myocardium.

Key words : ISO-HCl, Serum, AChE, GOT, GPT.

Introduction

Heart attack is one of the major causes of mortality among the populations of both developed and developing countries and is also prevalent in rural as well as urban areas in India. The diagnosis of myocardial infarction depends upon the characteristic electrocardiographic changes. The appearance of pathological Q-wave is virtually always specific for myocardial necrosis, but it may be absent unless the infarction is transmural. Therefore, the enzyme assays are important in the diagnosis of myocardial infarction. During myocardial necrosis there is rupture of cardiac muscles due to which number of enzymes are released but recently tissue specific hybrid CK-MB, troponin I, troponin t, LDH₁, and AST have been generally used as markers in the diagnosis of acute myocardial infarction (AMI) in human beings, in addition to ECG tracings. (Rasmussen *et al.*, 1979)

Isoproterenol hydrochloride is a well known drug to produce myocardial infarction (Lal and Rana, 1991; Sharma, *et al.*, 1987; Wexler and Greenberg, 1978). A very limited study has been so far made on ChE enzyme in the infarcted tissue or plasma, although cardiac muscles are richly innervated by cholinergic nerve fibres (Gaur and Kumar, 1993; Gaur *et al.*, 1999; Parveen and Kumar, 1994). During necrosis of myocardium, the AChE may be released and can be taken as a parameter for the diagnosis of MI.

Materials and Methods

The experimental animals included in the present study were *Columba livia* and *Rattus norvegicus*. In living condition six *C. livia* and six *R. norvegicus* were collected and acclimatised in laboratory according to their feeding habits and habitats. The human serum of about twelve patients was collected locally from Gandhi Medical College, Bhopal and Aayushman Heart Hospital, Bhopal. The effect of drug ISO-HCl was studied on activity of GOT, GPT and AChE and enzyme kinetics of AChE in serum of experimental animals. For experimental work in individual animals drug, ISO-HCl (85 mg/kg b.w.) was injected subcutaneously for two consecutive days. The GOT and GPT activity were determined by Reitman and Frankel (1957) method. For biochemical and enzyme kinetic of AChE, methods given by Augustinsson (1957) and Metcalf (1951) have been adopted, using AChI as a substrate. The AChE enzyme kinetic parameters K_m and V_{max} of control and experimental animal's serum were computed from Lineweaver and Burk (1934) plot according to Michaelis and Menten (1913) equation.

Results and Discussion

The GOT activity in the serum comparatively increased after the experimental production of MI from that of normal. The percentage increase of GOT activity was 110% in *C.*

livia and 115.38% in *R. norvegicus*. Human beings who suffered from MI also showed an increase in GOT activity by 78.57% to that of normal. The GPT also showed similar pattern in the activity. The percentage increase of GPT in serum of *C. livia* and *R. norvegicus* was 94.11% and 79.62%, respectively. In MI patient's serum the percentage increase of this enzyme was 56.00% (Table 1).

The AChE was also determined in normal and artificially produced MI in *C. livia* and *R. norvegicus* and in normal and MI patient's serum. It was noticed that the specific activity decreased in the homo-iothermal vertebrates after the production of MI and in naturally occurring MI patients. The percentage inhibition of AChE was -14.82% in *C. livia*, -19.21% in *R. norvegicus* and -15.68% *H. sapiens* (Table 1, Fig. 2).

The inhibitory constants of AChE, K_m and V_{max} in serum of control and ISO-HCl treated *C. livia* were $3.78 \times 10^{-3} M$ and $5.11 \times 10^{-3} M$ respectively. The V_{max} was found to be constant i.e. 1.0 A/mg protein / 30 min. However, in serum of control and experimental *R. norvegicus* the K_m value was $3.2 \times 10^{-3} M$ and $4.5 \times 10^{-3} M$ respectively. The V_{max} value was constant in both the cases i.e. 2.0 A/mg protein/30 min indicating that ISO-HCl has inhibitory effect for serum. In MI patients serum, the K_m value was $4.5 \times 10^{-3} M$ as compared to control value $2.76 \times 10^{-3} M$. The V_{max} value remained constant i.e. 1.0 A/mg protein/30min (Table 2, Fig. 1) showing that the inhibition is competitive in nature.

In control *R. norvegicus* the ventricular myocardium is richly innervated by large ramifying

Table - 1 : Effect of ISO-HCl (85 mg/kg b.w.) on enzyme activity of serum of homo-iothermal vertebrates compared with human serum of MI patients.

Animal / Human beings	Control / treated	Enzyme activity					
		GOT (mM/ml)		GPT (mM/ml)		AChE (μM /mg protein/hr)	
		Activity	% Increase	Activity	% Increase	Activity	% Inhibition
<i>Columba livia</i>	C	0.20 ± 0.183	-	0.17 ± 0.156	-	1.62 ± 0.591	-
	T	$0.42 \pm 0.169^*$	110.00	$0.33 \pm 0.138^*$	94.11	$1.38 \pm 0.412^{****}$	-14.82
<i>Rattus norvegicus</i>	C	0.65 ± 0.216	-	0.54 ± 0.246	-	0.932 ± 0.502	-
	T	$1.40 \pm 0.210^{***}$	115.38	$0.97 \pm 0.199^{**}$	79.62	$0.753 \pm 0.187^{****}$	-19.21
<i>Homo sapiens</i>	C	1.40 ± 0.472	-	0.50 ± 0.186	-	0.555 ± 0.192	-
	T	$2.50 \pm 0.497^{***}$	78.57	$0.78 \pm 0.192^*$	56.00	$0.468 \pm 0.173^{****}$	-15.68

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

**** Non significant

Table - 2 : Effect of ISO-HCl (85 mg/kg b.w.) on kinetics of serum AChE of homo-iothermal vertebrates and compared with human serum of MI patients.

Animals	Control/ISO-HCl	$K_m \times 10^{-3} M$	V_{max} A/mg protein/ 30 min
<i>Columba livia</i>	0	3.78 ± 0.824	1.0
	ISO-HCl	$5.11 \pm 1.33^*$	1.0
<i>Rattus norvegicus</i>	0	3.2 ± 1.029	2.0
	ISO-HCl	$4.5 \pm 0.824^*$	2.0
<i>Homo sapiens</i>	0	2.76 ± 0.664	1.0
	MI patients	$4.5 \pm 1.005^{**}$	1.0

* $P < 0.05$ ** $P < 0.01$

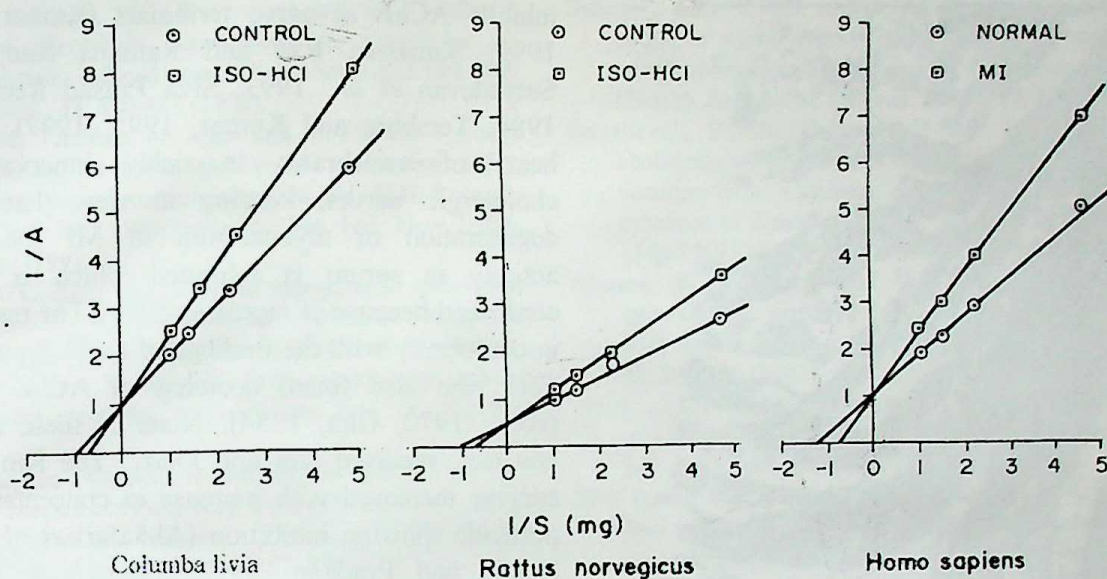


Fig. 1 : Lineweaver Burk plot of inhibition of serum AChE by ISO-HCl (48 hrs). (S) is the substrate concentration of ACh. Each point is the mean of six assays.

nerve fibres (Fig. 3). After administration of ISO-HCl, heart wt/body wt. ratio increased in the experimental animals. Besides it also showed irregular heart beat (arrhythmias). The infarcted cardiac muscle showed necrosis in the sub endocardial muscle fibres (Fig. 4). In the myocardial bundles lysis had been noticed at several places. However, these myocardial bundles were replaced by

mononuclear cells and confirmed the observations of Kaur *et al.* (1995), Lal and Rana (1991), Sharma *et al.* (1987) and Singh *et al.* (1988).

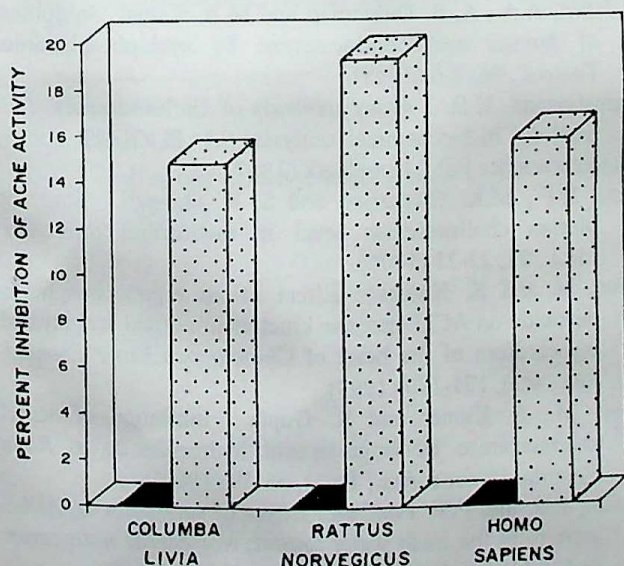


Fig. 2 : Percent inhibition of AChE activity of serum of homoiothermal vertebrates exposed to isoproterenol hydrochloride (85 mg/kg body wt) for 48 hours and compared it with *Homo sapiens* of myocardial infarction patients.



MYO : Myocardium; NF : Nerve fibres
RV : Right ventricle.

Fig. 3 : Photomicrograph of the heart of *Rattus norvegicus* showing large ramifying nerve fibre innervating the ventricular myocardium. Longitudinal section X 100. Ungewitter's silver stain.



LV - Left ventricle; MNC - Mononuclear cells;
MYO - Myocardium; Ne - Necrosis

Fig. 4 : Photomicrograph of the heart of *Rattus norvegicus* showing necrosis and mononuclear cells in the left ventricular myocardium. Longitudinal section $\times 100$. Haematoxylin counter stain with eosine.

The LDH and its isoenzyme, GOT and GPT are important routine clinical enzymes in the diagnosis of MI in human beings. The creatine kinase-muscle brain (CK-MB) is now a marker enzyme but is positive in the first 6 hrs. A number of investigators reported the increase of GOT and GPT in the artificially produced MI by ISO-HCl in rats and in MI patients (Kaur *et al.*, 1995; Singh, 1988).

In the present study the MI was produced in *C. livia* and *R. norvegicus* by injecting 85mg/kg b.w. ISO-HCl (Kaur *et al.*, 1995; Lal and Rana, 1991). The increase in the activity of GOT and GPT shows similar pattern and is in agreement with earlier authors and similar in case of human beings, both in the normal and in MI patients. It is suggested that in birds the level of this enzyme also increases as in the case of mammals.

The AChE activity during the process of myocardial necrosis has not been studied in detail. The literature regarding the AChE activity and its inhibition deals with pesticide toxicity as pesticide

inhibits AChE at nerve terminals (Kumar *et al.*, 1999; Samasiva Rao and Ramana Rao, 1989; Satyadevan *et al.*, 1993; Siva Prasad Rao *et al.*, 1984; Tembhre and Kumar, 1995, 1997). As the heart of vertebrates is richly innervated by cholinergic nerves, keeping in view that during degeneration of myocardium in MI the AChE activity in serum is inhibited which is further confirmed because of increase of Km. The results are in conformity with the findings of the authors in this field who also found decrease of AChE activity (Basu, 1970, Oka, 1954). None of these authors, however, reported Km and V_{max} . The Km of this enzyme increased with increase in concentration of pesticide showing inhibition (Al-Jafari *et al.*, 1995; Hande and Pradhan, 1990; Jagota, 1992). The present study concludes that there is increase of Km value, in the artificially produced MI and in MI patient's serum. It is suggested that these parameters are important in the diagnosis as well as for predicting prognosis in case of MI, in addition to tissue specific CK-MB. The present study corroborates our previous observations on the heart of fish subject to injury in the ventricular myocardium (Gaur and Kumar, 1993).

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Chemical components of heartwood and sapwood of common Yew (*Taxus baccata* L.)

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Abstract : Cell-wall components and solubility characteristics of the heartwood and sapwood of *Taxus baccata* L. were determined by methods of wood analysis and the differences between heartwood and sapwood were established. When we observe the data obtained, it is seen that the amount of extractive material found in the heartwood is substantially higher than the sapwood. The extractive material in *Taxus baccata* L. is originated from the hidden epithelial cells surrounded by resin canals.

Key words : Common Yew, *Taxus baccata* L. Heartwood, Sapwood, Chemical composition of wood.

Introduction

It is well known that taxol, extracted from *Taxus baccata* L. is used in the treatment of cancerous patients. Taxol is effective in the breast and lung cancers and it was used in 1994, in the treatment of breast, uterus and lung cancers, which did not respond to classical chemotherapy (Kavalali, 1999). The chemical composition of components of *Taxus baccata* L. was determined using standard methods of wood chemistry and the differences between the heartwood and sapwood have been established.

Materials and Methods

In the studies carried out on *Taxus* species, it is known that there is only one native *Taxus baccata* L. species in Turkey. It is found that this species grow in our forests in small groups at 600-2000 m elevations, and that they expand from Trakya Eastern Anatolia to Northern Iran (Özgüroğlu and Berkarda, 1999; Baytop, 1999). They are found in small groups of Vize, Ayancik, Yenice (Zonguldak), Karabük, Düzce, Bolu, Demirköy forests in the Black Sea region and in Western Anatolia from Kazdağı Mountains (İda) to Sultan Mountains, in the coastal forests of Mediterranean and in Amanos Mountains in Hatay (Yaltirik and Efe, 1994).

Taxus baccata L., used in this study as a material was taken from Demirköy Forest and named as sample D. It was 240-250 years old and had a

diameter of about 34.5 cm; three sample disks, of which were taken from the butt, 4-5 ft. height and the top of trees.

When the wood samples are prepared, firstly the barks removed and then the sap and heartwood are separated. They are chipped with a thickness 2-5 mm and then they are ground in a Wiley laboratory type mill according to the TAPPI os-75 standards and the wood flour obtained. Screening procedure has been carried out by a shaking sieve, taking the parts, which pass through the 40 mesh and remain on the 100 mesh and they are kept in glass jars to obtain equilibrium moisture. After determination of moisture by using TAPPI Test Methods (1992-1993) the following determinations have been carried out ash (T 211 om-85), %1 NaOH solubility (T 212 om-85), hot water solubility (T 207 om-85), alcohol solubility (T 204 om-88), holocellulose (Wise and John, 1952), α - cellulose (Wise *et al.*, 1946), lignin (Runkel and Wilke, 1951), pentosan (Bethge, 1964).

Results and Discussion

Solubilities of sapwood and heartwood from Demirciköy and the components of the cellular wall were given in Tables 1 and 2, respectively. On the other hand, some values about sample A, E, and P (given references) of *Taxus baccata* L sapwood and heartwood were also given in the Table 3, 4 and 5 for comparisons with *Taxus baccata* L. from Demirciköy.

Table – 1 : The solubilities of *Taxus baccata* L. from Demirciköy

	Ethanol benzene (%)	Alcohol (%)	Petroleum-Ether (%)	Cyclo Hexanane (%)	%1 NaOH (%)	Hot water solubility	Ash %
Heartwood D	19.11	0.3242	0.5122	1.376	26.13	6.36	0.1578
	19.34	0.3232	0.552	1.195	26.6	6.21	0.1468
Average	19.23	0.3219	0.5321	1.286	26.4	6.29	0.1523
Sapwood D	3.04	0.16	0.2023	0.0063	15.69	5.15	0.3191
	3.14	0.15	0.1504	0.0063	15.82	5.36	0.3163
Average	3.09	0.155	0.1764	0.0063	15.76	5.26	0.3177

Table – 2 : The cellular wall components of *Taxus baccata* L. from Demirciköy.

	Holocellulose (%)			α cellulose (%)	Lignin	Pentosan (%)	
	Lignin + holocell (1)*	Residual lignin (2)*	Calc. holocellulose (1-2)*			In the wood	Holo cell
Heartwood	77.99	6.39	71.60	53.15	30.22	9.75	11.40
	77.47	6.37	70.10	53.25	30.00	9.85	11.20
Average	77.73	6.38	70.35	53.20	30.11	9.80	11.30
Sapwood	80.26	6.35	73.95	53.36	31.55	10.08	11.48
	80.24	6.55	73.80	53.24	31.5	10.02	11.56
Average	80.25	6.45	73.88	53.30	31.53	10.04	11.52

(1)*With residual lignin holocellulose

(2)*Residual lignin

(1-2)*Calculated holocellulose

Table – 3 : Some characteristics of sample *Taxus baccata* L. (Uslu, 1997)

Restrict	Sample number	Wood average age	Diameter (cm)	Symbol
Amasya	3	53-79	14-17.5	A
Espiye	3	195-243	25.5-28	E
Pazar	3	86-155	15.5-34	P

Table – 4 : The solubilities of Some *Taxus baccata* L. (Uslu, 1997).

	Ethanol benzene (%)	%1 NaOH (%)	Hot water (%)	Ash (%)
Heartwood A	10.23	18.77	9.33	0.49
Heartwood E	20.58	26.58	16.20	0.56
Heartwood P	15.10	21.27	12.55	0.36
Sapwood A	2.73	10.27	2.83	0.32
Sapwood E	2.34	10.50	2.66	0.40
Sapwood P	2.12	12.30	3.10	0.29

Table - 5 : The cellular wall components of Some *Taxus baccata* L. (Uslu, 1997).

	Holocellulose (%)	α -cellulose(%)	Lignin(%)	Cellulose(%)
Heartwood A	63.38	40.35	28.16	48.55
Heartwood E	54.49	34.47	26.05	41.66
Heartwood P	55.76	35.84	28.79	45.94
Sapwood A	69.07	45.29	29.86	52.16
Sapwood E	66.65	42.94	31.12	53.20
Sapwood P	66.49	41.69	30.37	52.98

As is seen in Table 1 the mean ethanol-benzene solubility is 19.23 % in the heartwood and 3.09% in the sapwood from Demirciköy, the characteristic feature of the hardwood trees is to form resin in a normal or pathologic way. The amount of extractive material of the heartwood is 6 times more than that of the sapwood. It is suggested that the amount of extractive material found in *Taxus baccata* L. is originated from the hidden epithelial cells surrounded by resin canals. Heartwood from Demirciköy, the ethanol- benzene solubility when compared with the others (Table 3, 4 and 5), it has been seen that in Table 4 it is higher than the others except for heartwood from Espiye. They are thought that the difference in Amasra, Pazar and Espiye are caused by their ages. *Taxus baccata* L. from Espiye are older than those Amasra and Pazar (Table 3). Sapwood from Demirciköy, the ethanol-benzene solubility has been found higher when compared with the others .

It is thought that the increase in the solubility of carbohydrate of lower molecular weight, degraded cellulose, polyoses, and particularly pentosan can be explained by the effect of 1 % NaOH (in the heartwood from Demirciköy 26.4%, Sapwood from Demirciköy 15.76% (Table 1).

In acidic medium, NaClO₂ delignification increases the solubility of lignin. The determination of standard lignin (the residual lignin) in holocellulose, always gives a low yield. Holocellulose, which is calculated by the subtraction of the residual lignin (given 1*, 2* and 1-2* in the Table 2), exceeds the 100% together with the amount of lignin.

When Table 2 studied, it is found that the cellular wall materials of the sapwood such as

cellulose (α -cellulose), polyoses (pentosan) and lignin are higher amounts than those of the heartwood. The amount of α -cellulose is 53.30% in sapwood and 53.20% in heartwood and this gives the typical rates of cellular wall components (Table 2). But in the extractive materials in the heartwood are the opposite. The amounts of ethanol-benzene, alcohol, petroleum ether, cyclo hexanol, %1 NaOH and hot water solubility are higher amounts than those of the sapwood (Table 1). Uslu (1997) suggests that these values are associated with the typical sapwood values (Table 4 and 5). Also, the literature proves these result (Fengel and Wegener, 1988; Balaban *et al.*, 1999). The amount of pentosan has been studied separately in the wood and holocellulose. When the amount of pentosans in the wood and in holocellulose was compared with each other, it is seen that they give almost the same (Table 2). It is thought that the difference in the wood and holocellulose is caused by the polysaccharides, which are dissolved during delignification.

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Short communication

Effect of nicotine (plant extract) on sex-ratio of *Drosophila melanogaster* (Meigen)

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Abstract : Experiments were conducted by using nicotine (plant extract) for its toxic effects on *Drosophila melanogaster*, LC_{50} estimated is $2.9552 \mu\text{l}/100 \text{ ml}$. Studies revealed that nicotine affects adult emergence of males and females (sex-ratio) of mutant form (Yellow) of *Drosophila melanogaster*.

Key words : Nicotine, *Drosophila melanogaster*, Toxicity.

Introduction

Several plant products were tried to find out whether they could be used as alternatives to the toxic synthetic pesticides as they are less persistent in nature and are non-hazardous to humans. The toxic effect of different plant extracts has been reported by several workers such as Akhter and Bahadur, 2002; Barakat *et al.*, 1985; Sagar and Sehgal, 1997; Choudhary, 2002a,b,c; Malik *et al.*, 1996. The purpose of the present study was to evaluate the toxicity of nicotine (plant extract) on sexes of mutant form (Yellow) of *Drosophila melanogaster*.

Materials and Methods

The pure strain of Yellow *Drosophila melanogaster* was obtained from *Drosophila* Stock Centre, School of Life Sciences, Devi Ahilya Vishwavidyalaya, Indore. The pure culture was maintained under the laboratory conditions at a temperature $25 \pm 5^\circ\text{C}$ and on a standard food medium consisting of corn flour 1.7 g, agar 2g, sugar 12 g, yeast 3 g, nepagin 1g (anti-bacterial), propionic acid 1 ml (anti-fungal), 70% alcohol 1 ml and distilled water 360 ml for one unit (10 experimental bottles) which was prepared according to the method described by Aijaz *et al.* (1987). The flies of this culture were used for experiment after 5-6 generations, when they were fully acclimatized to the laboratory conditions.

The LC_{50} ($2.9552 \mu\text{l}/100 \text{ ml}$ food) was calculated first and then a sub-lethal ($0.312 \mu\text{l}/100$

ml food) dose was selected. In order to determine the effectiveness of the test compound, the selected sub-lethal dose was mixed in culture medium according to the method described by Dhingra *et al.* (1988) and different cross combinations (three sets for each combination) (i) $T\text{♀} \times U\text{♂}$, (ii) $T\text{♀} \times T\text{♂}$, (iii) $U\text{♀} \times T\text{♂}$ were examined against the control set bottles (iv) $U\text{♀} \times U\text{♂}$ [T=Treated, U=Untreated]. Flies were etherized for separation of males and females in the ratio of 1 : 1 and were then transferred in the culture bottles (*vide supra*). Eggs were collected by Delcours procedure (Delcours, 1969). Eggs of each set were allowed to undergo development in the same culture bottles and observations were thus made of fecundity and adult emergence. The flies emerged were counted and sexed every day from the first day upto the last day of eclosion.

The collected data were analyzed statistically by log-dose / probit regression line method (Finney, 1971). The statistical calculation of mean, standard deviation and standard error were based on the biological statistics by Fisher and Yates (1963). The test of significance was made using simple t-test.

Results and Discussion

It was observed that the emergence of sexes of the second set shows more reduction (Table) followed by first and third sets respectively as compared to the control set.

The result shows that the compound causes considerable affect as the fecundity has been

Table – 1 : Sex-ratio of experimental mutant (Yellow) *D. melanogaster* after nicotine (plant extract) intoxication.

S. No.	Sets	No. of adults emerged (Mean + S.E.)	Percent sex-ratio (Mean + S.E.)
1.	T♀ × U♂	253.00 ± 2.12	0.78 ± 1.33**
2.	T♀ × T♂	196.66 ± 9.22	0.65 ± 0.76***
3.	U♀ × T♂	307.00 ± 11.67	0.86 ± 1.32*
4.	U♀ × U♂	347.66 ± 12.45	0.98 ± 0.52

***statistically very highly significant, **statistically highly significant and *statistically significant.

observed to show reduction. The reduction may be due to inhibitory effect of the nicotine on the gonadal development (Choudhary, 2002). Sexual emergence shows significant decreases as compared to control. The females out number the males yet the females are more susceptible because they cannot tolerate the effect of given insecticide. It also signifies a positive relationship between the natality and mortality of a particular sex. Sex-ratio is one of the adaptive traits of any population which determines the rate of increase or decrease of a sexual population in an environment. Environmental factors such as temperature, food, space etc. are also known to affect the sex-ratio. The sex-ratio shows significant decrease. In the present finding, the sex-ratio has been reduced due to the test chemical only, because all other factors were not variable but were kept constant. The reduction in sexual emergence may be due to the affect of test chemical on the apolytic process of the insect.

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Author Index

(Page-wise)

J. Environ. Biol., 24(1 to 4) 2003

A

Acar, Cengiz	415
Adebiyi, A. A.	309
Adeleye, I. A.	309
Aery, N. C.	117
Agrawal, Meena	177
Ahmad, A.	141
Ahmad, Iqbal	141
Ahn, Kyu-Hong	29
Ajungla, T.	461
Akhtar, M.	9
Al-Ayed, M. I.	271
Altun, Lokman	415
Amoji, S. D.	81, 165
An, Kwang-Guk	29, 147, 229
Anand, J. P.	321
Angiras, N. N.	357
Asokan, S.	477
Atluri, J. B.	295
Avsar, Mahmut D.	155
Azam, Z. M.	141

B

Babu, K. Lenin	223
Baghel, Vinay Singh	349
Baig, M. I. A.	173
Baig, M. M. V.	173
Bakare, A. A.	429
Bansal, S. K.	113
Barat, Sudip	339
Başkent, E. Zeki	415
Bhadra, Bhaskar	125
Bhatia, A. L.	369
Bhattacharjee, S.	395
Bilgili, Ertuğrul	415
Bindu, Alex	449
Biradar, Pulikeshi M.	81
Biradar, V. A.	165
Bohra, N. K.	213
Bounias, M.	1

C

Caglar, S.	315
Chakrabarti, Nandini	289
Chakraborty, Ranadhir	125
Chandran, R.	103, 201
Chandrasekar, R.	381
Chandrashekar, J. S.	223
Choudhary, Nisha	305
Choudhary, Shamim	493
Cicek, A.	281

D

Das, P. K.	465
Dash, Kalpana	265
David, M.	69
Dawra, J.	331
Deena, M. J.	211
Degloorkar, N. M.	91, 205
Dkhar, M. S.	461
Doran, I.	315, 437
Dwivedi, B. K.	55

E

Evrendilek, Fatih	241
-------------------	-----

F

Farmer, C.	411
------------	-----

G

Gaur, Madhavi	483
Gökbülak, Ferhat	45
Gopal, Krishna	349
Gupta, D. K.	9

H

Haffor, S. A.	271
---------------	-----

Hayat, S.	141
Hazarika, Ranjit	77
Hosetti, B. B.	193
Hou, Shaofan	423

I

Inam, A.	141
Ingle, S. T.	63

J

Jain, A. K.	265
Jain, S.	331
Jha, Prithwiraj	339
Joshi, S. C.	305

K

Kanakasabai, R.	477
Karpagam, G.	201
Kaushik, A.	331
Kaya, Z.	315, 437
Khan, S. Ajmal	103, 201
Khanna, Shalini	391
Khokale, D.	253
Kobayashi, Katsumi	39
Koparal, A. S.	281
Krishnamoorthy, P.	327
Krishnatrey, Richa	161
Kumar, Santosh	483
Kumarasinghe, N. C.	359

L

Lakshmanaperumalsamy, P.	373
Lee, Mi Young	17
Li, Hairong	423
Li, Yonghua	423

M

Madhavi, A.	187
Maiti, S. K.	465
Manda, K.	369
Mane, V. P.	173
Mastan, S. A.	405
Mathur, Neera	161

MERTOĞLU-ELMAS, Gülnur	489
Minija, J.	211
Miura, Naoyuki	39
Mohamed, M. Aneez	381
More, D. R.	173
More, P. R.	91, 205
Mosuro, A. A.	429
Mukherjee, A.	265
Mukherjee, A. K.	395
Mukherjee, Shriparna	125
Mukherji, S.	289
Mukhopadhyay, A.	471
Mushigeri, S. B.	69

N

Nagarajan, R.	477
Namdas, S. B.	63
Nanda, Ashis K.	125
Neelima, P.	453

O

Osibanjo, O.	429
--------------	-----

P

Pal, Amit	9
Pandey, A. K.	265
Pandey, G. C.	55
Park, Seok Soon	29
Pathak, Shipra	161
Pradhan, B.	471
Prashanth, M. S.	69
Pulikeshi, M. B.	165
Punzo, F.	23, 411
Purohit, D. K.	213

Q

Qureshi, T. A.	405
----------------	-----

R

Rai, U. N.	9
Rajasegar, M.	95
Rajeswary, S.	401
Rajput, S.	253

Rajurkar, S. R.	91, 205
Ramalingam, V.	401
Ramana, S. P. Venkata	295
Rana, D. K.	117
Rana, K. S.	345
Rana, S. V. S.	135
Rao, A. Prasad	187
Rao, J. V.	445
Ray, S. K.	465
Razdan, Twinkle	345
Reddi, C. Subba	295
Reddy, K. Jaganmohan	453
Rekha, K.	449

S

Saha, K.	265
Sambasivam, S.	103, 201
Samiullah	141
Saravanan, T. S.	381
Sastry, L. V. M.	261, 445
Sato, Eiji	39
Savithamani, K.	373
Saxena, P. N.	345
Selvamurthy, W.	321
ŞEN, Bahtiyar	437
Shagoti, U. M.	165
Sharma, Anjana	253
Sharma, G. D.	461
Sharma, K. P.	161
Sharma, Meenakshi	305
Sharma, P.	331
Sharma, Subhasini	161
Sherpa, P. W.	471
Shibata, Kiyoshi	39
Shinde, D. N.	63
Shinde, L. P.	173
Shridhar, D.	253
Singh, Jaswant	349
Singh, K. P.	357
Singh, Karam V.	113
Singh, P. K.	107
Somashekar, R. K.	223
Srivastava, C. N.	391
Srivastava, M. M.	391
Srivastava, Neera	177
Srivastava, Shalini	391
Sundramoorthy, M.	381

Suryavathi, V.	401
----------------	-----

T

Tajo, A.	211
Tan, Jian'an	423
Teresa, M. V. Merlee	449
Tewari, R. K.	107
Thakur, Lalan	321
Tharavathi, N. C.	193
Thiyagesan, K.	477
Thoppil, J. E.	211
Tilak, K. S.	261, 445
Tonguc, Fatih	155
Turna, İbrahim	415
Tyagi, Anupama	177

U

Uezato, Tadayoshi	39
Urchin, Christopher G.	29

V

Vadlamudi, V. P.	91, 205
Vaithinathan, S.	327
Veeraiah, K.	261, 445
Verma, Pramod	305
Verma, Yeshvandra	135
Vimaladevi, V.	401
Vivekanandhan, G.	373

W

Wang, Wuyi	423
Wenhai, Chen	181
Wu, Yi-Xin	39

X

Xinjiao, Dong	181
---------------	-----

Y

Yang, Linsheng	423
Yi, Ki Wan	17
Yilmaz, Murat	415

